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Allelic Expansion Underlies Many Genetic Diseases

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For decades, most genetic diseases were little more than medical curiosities. The genes harboring the responsible mutations, the nature of the mutations, and the mechanisms by which the mutations produced clinical disease were either unknown or poorly understood. However, this is now rapidly changing. Nowhere is our accelerated understanding more evident than in disorders that result from instability of trinucleotide repeats of DNA.

Indeed, **observations over the past 4 years have revealed that mutations involving instability of trinucleotide repeats, or DNA tracts of tandemly repeated 3-bp units, are responsible for numerous human genetic disorders** (Table 1, page 2).[1] Since the instability usually involves an increase in the number of repeats in only 1 of a person's 2 alleles for a given gene, **the phenomenon is often called "allelic expansion."** Delineation of this phenomenon, combined with the recent discovery that mutations in DNA repair genes leading to familial cancer syndromes may contribute to such genetic instability, has increased both the awareness of and interest in these repeat DNA sequences, and in the consequences of their instability. In this article, some aspects of the data regarding instability of simple repeats during DNA replication and genetic transmission are reviewed and current hypotheses regarding mechanisms leading to this remarkable phenomenon are presented.

Among the trinucleotide repeat disorders, **2 general classes of instability of the repeat sequences exist.** The **first class** has mutations leading to only small-scale alterations of up to 2-fold the original length in repeat size; in the **second class**, both small-scale and quite large-scale changes of as much as 20-fold occur (Table 1, page 2). **No mechanism has been demonstrated to account for either type of mutation.** While it is tempting to view these 2 general types of mutations as different mechanistically, there is no direct evidence as yet to support this view.

DISEASES RELATED TO SMALL-SCALE CHANGES

Disorders characterized by exclusively **small-scale instability** include **spinobulbar muscular atrophy** (SBMA), **Huntington disease** (HD), **spinocerebellar ataxia type 1** (SCA1), **dentatorubral pallidoluysian atrophy** (DRPLA), and **Machado-Joseph disease** (MJD).[2-7] Each disorder exhibits mutations that change the number of CAG repeats. Since the trinucleotide-CAG codes for glutamine, these mutations are presumed to alter the length of polyglutamine tracts in the respective gene products, which leads to degeneration that is specific for subsets of central or spinal neurons. These disorders recur when the expanded repeat sequence is transmitted from parent to child. Little or no variation in repeat size is observed in somatic tissues of an individual, ie, there is no somatic mosaicism. It is tempting to attribute the instability observed during parent-to-child transmission to mutations occurring during meiosis, and to attribute the instability observed during somatic growth of an individual to mutations occurring during mitosis. However, it is not clear whether the alterations observed before conception occur during meiosis or in the mitotic divisions that precede or even follow sperm or egg production.

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Small-scale expansions can reach a doubling of the repeat number, and occasionally reductions in repeat number are found. Expansions vastly exceed reductions, and the likelihood and magnitude of change are related to whether they are inherited from the mother or the father. In general, paternal transmissions result in greater changes in size and more frequently involve increases. Measurement of expanded repeat lengths in sperm demonstrate directly that these increases occur in the germ line of the father rather than in the embryo. It also is known that **patients who carry large repeat lengths often have early onset of their disorder**.[4-9] These phenomena help explain the greater likelihood of juvenile onset of HD, SCA1, and DRPLA when the expansion is inherited from affected fathers rather than affected mothers. The propensity for expansion provides a clue in considering the mechanism of instability.

The alleles containing the maximum number of repeats found in patients with SBMA is 62. In HD, the maximum number of repeats found is 121. These sizes are below the low end of repeat sizes in disorders subject to large-scale changes (Table 1). This size restriction may reflect the presence of the CAG repeat in the protein-coding regions of these genes. Alteration might simply abolish the functions of such proteins. However, this does not seem to be the case, since mutations known to abolish function of these proteins produce different clinical manifestations compared with those involving allelic expansion. As noted earlier, individuals carrying expanded repeats in this general class rarely show mosaicism, ie, multiple sizes of alleles within a tissue. This is in marked contrast to what is found in individuals carrying the large amplifications, where multiple sizes of alleles within a tissue occur.

DISORDERS RELATED TO LARGE-SCALE CHANGES

In fragile site-related mutations, such as in the fragile X syndrome[10-12] and in myotonic dystrophy,[13-15] increases in repeat number as much as 20-fold have been observed in a single parent-to-child transmission.

Table 1
Characteristics of Unstable Trinucleotide Repeats Identified to Date

Disease	Chromosome	Locus	Location in Associated Gene	Repeat	Size in Normal	Size in Carrier	Size in Affected	Change in Gene Function
Kennedy disease (SBMA*)	Xq11-12	AR	Exon 1	CAG (gln)	12-34	–	40-62	Gain
Huntington disease	4p16.3	HD	Exon 1	CAG (gln)	6-37	–	35-121	Gain
Spinocerebellar ataxia type 1	6p22-23	SCA1	Exon 8	CAG (gln)	6-39	–	41-81	Gain
Dentatorubral pallidolusyian atrophy	12p12-13	DRPLA	Exon 5	CAG (gln)	7-34	–	54-70	Gain
Machado-Joseph disease	14q32.1	MJD	Internal exon?	CAG (gln)	13-36	–	68-79	Gain
Fragile X syndrome	Xq27.3	FRAXA (FMR1)	5' untranslated	CGG	5-52	43-200	230->2,000	Loss
Dystrophia myotonica	19q13.3	DM	3' untranslated	CTG (CAG)	5-37	44, 46	50->2,000	RNA stability?
Mental retardation?	Xq27.3	FRAXE	??	GGC (CGG)	6-25	116-133	200->850	??
(None)	Xq28	FRAXF	??	GGC (CGG)	6-29	–	300-500	??
(None)	16p13.11	FRA16A	??	GGC (CGG)	16-49	–	1,000-2,000	??

Small-scale polyglutamine (gln) disorders are in the top half of the table, and large-scale mutations are in the bottom half. Repeat sequences are listed in the coding strand and frame, where known, or as reported by the authors of the study; however, only 2 triplets (CAG and CGG) are represented.

* Spinal and bulbar muscular atrophy

It is possible that **large-scale expansions** result from the same mechanism as the small-scale changes. However, in these instances they **are the consequence of many rounds of expansion during the DNA replication preceding meiosis or mitosis occurring in the early embryo**. In support of this notion is the finding of significant levels of mosaicism in the absence of ongoing instability in the fragile X syndrome, suggesting an early embryonic expansion limited to somatic tissues.[16] Evidence in this syndrome also suggests that the male germ line is spared the expansion event. However, it should be noted that sufficient differences exist in the characteristics of the large-scale expansions to raise questions as to whether one mechanism is common to all. For example, such expansions occur only with maternal transmission in fragile X syndrome, while both maternal and paternal transmissions can result in large increases in myotonic dystrophy. Reductions are observed rarely in myotonic dystrophy and in fragile X syndrome.

LESSONS FROM OTHER REPEAT SEQUENCES

Because of their utility in genetic mapping, a vast number of repeats of the dinucleotide CA have been isolated and characterized. The fact that their sizes are polymorphic, ie, their sizes vary in the population, indicates that they are unstable. Moreover, the likelihood and degree of this polymorphism correlate with the length and purity of the repeats. However, the instability of CA repeats is largely historical, since in today's population they are genetically transmitted with high fidelity. Indeed, this fidelity allows their use as genetic markers. Mutations can be found in CA repeats, and the frequency has been estimated from family studies at $\sim 5 \times 10^{-4}$ per gamete per generation.[17] Direct analysis of cell lines provides a measurement of $\sim 1 \times 10^{-5}$ per cell per generation for a CA_{17} repeat.[18] While these values are significantly higher rates of mutation than are found in DNA sequences outside of a simple repeat sequence, they are well below the rates found in the trinucleotide repeat disorders mentioned earlier ($\sim 10^{0}$).

Mutations in CA repeats involve additions or deletions of one repeat unit or a few repeat units, analogous to the small-scale changes found in trinucleotide repeats described previously. The frequency of mutation may relate to length, as is suggested by the higher levels of polymorphism found in the longer repeats. **Most CA repeats are less than 30 repeats in length (60 bp), while the instability found in trinucleotide repeats becomes significant above 35 repeats (100 bp).** In accord with this is the observation of greater mutation rates in tetranucleotide repeats assayed in family studies (2.1×10^{-3}).[17] Tetranucleotide repeats tend to be longer overall than CA repeats (40 to 100 bp).

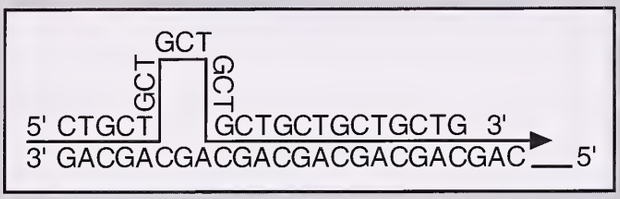
MISMATCH REPAIR PROCESSES

Recent efforts to characterize familial cancer syndromes have identified **defects in repair of DNA in which bases are mismatched during replication**. These are termed **mismatch repair processes**. For example, Lynch syndrome, or hereditary nonpolyposis carcinoma of the colon (HNPCC), was mapped to sites on chromosomes 2 and 3 by analyzing how tumors were inherited in families and detecting loss of heterozygosity (LOH) in tumors. The latter refers to the fact that certain genes, known as tumor suppressor genes, need to be present in at least one copy (heterozygosity) to prevent tumors. LOH for such protective genes is often found in tumors. The LOH analyses utilized CA repeat DNA markers, and the number of repeats was often found to have changed in the tumors compared with normal tissues from the same individual. These alterations are similar to the small-scale changes found in the trinucleotide repeat disorders, involving increases and decreases by one or a few repeat units. The instability found in HNPCC tumors suggested a possible role for defective mismatch repair in these families, since mutations of mismatch repair pathways in yeast (*Saccharomyces cerevisiae*) lead to similar instability of dinucleotide repeats. Indeed, mutations in 4 human genes equivalent to bacterial mismatch repair genes located on chromosomes 2, 3, and 7 have been found in Lynch syndrome families,[19-23] and cell lines derived from such individuals exhibit defects in mismatch repair. **It is postulated that defective mismatch repair allows accumulation of mutations that promote tumorigenesis.** Observed changes in the number of CA repeats are in essence a side effect of the repair defects, but this offers insight into the mechanism responsible for these conditions.

SLIPPERY DNA

One mechanism for generating mismatched DNA involves slipped mispairing or slippage of repeated DNA sequences during replication. Figure 1 (page 4) shows generation of slipped structures. When these errors occur during DNA replication in yeast and *Escherichia coli*, they are recognized and repaired by the mismatch repair system. When unrepaired, such structures generally result in increases or decreases of one or a few slipped repeat units, resembling the small-scale changes seen in trinucleotide repeat disorders. Slipped mispairing is enhanced significantly by greater length and purity of repeat sequences, correlating with the increases seen in trinucleotide repeat instability. **It is likely that the small-scale changes result from slipped structures that are not adequately repaired**. There is no evidence to suggest that mismatch repair is defective in families with trinucleotide repeat disorders. In fact, the preponderance of the

Figure 1
Example of Slipped Mispairing During DNA Replication



DNA sequences with repetitive elements are found to have a higher likelihood of exhibiting slipped structures, which can result in both increases and decreases in repeat number. This mechanism has a role in trinucleotide repeat instability, which may be enhanced by the ability of the CTG and CGG sequences to adopt alternative DNA structures, helping to stabilize slipped structures.

evidence suggests otherwise. However, the peculiar developmental timing and tissue location of these changes may implicate certain times and locations where mismatch repair is ineffective, such as early in embryogenesis or in the germ line.

WHY INCREASES?

The excess of DNA repeat expansion over contraction in disorders characterized by trinucleotide repeats is not consistent with the model of slipped strand mispairing. Theoretically, slippage should be possible on both the newly synthesized and the template strand, resulting in both expansion and contraction. However, **recent data** demonstrating polar variation in the fragile X repeat **suggest a higher frequency of mutation in the newly replicated strands**. If newly synthesized fragments on the lagging strand (Okazaki fragments) are more prone to these changes, it might be expected that increases would be found more frequently, and that the direction of replication would affect the mutability and location of mutations.

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WHY ARE CGG AND CAG PREDOMINANTLY AFFECTED?

If length alone is the determinant of propensity of instability, then other simple sequence repeats of similar lengths might be expected to show similar levels of instability. There are no reports of mutation rates of similar levels in other simple sequence repeats, although the number of loci that would meet the criteria and that have been thoroughly investigated is not large. **The limitation to these sequences suggests specific structural features of these repeats that contribute to the mechanism of instability**.

MODELING THE LARGE-SCALE CHANGES

Slipped mispairing is not a compelling explanation for the large increases found in the fragile site syndromes and myotonic dystrophy. While multiple rounds of expansion could account for these events, it seems likely that a different mechanism is operative here. A number of models have been proposed,[10] and in the absence of an experiment system to reproduce this behavior, it is difficult to test these models.

CONCLUSION

Mismatch repair defects play a significant role in both human tumor genesis and some of the dramatic variations of the genomes associated with human genetic disease. As demonstrated by the number of diseases listed in Table 1 (page 2), this association can be anticipated to account for additional diseases that are not currently classified as resulting from the mismatch repair process. As the process is further studied we can anticipate understanding better the genetic variations in the normal and diseased states.

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Genetics of 3β-Hydroxysteroid Dehydrogenase Deficiency Disorder

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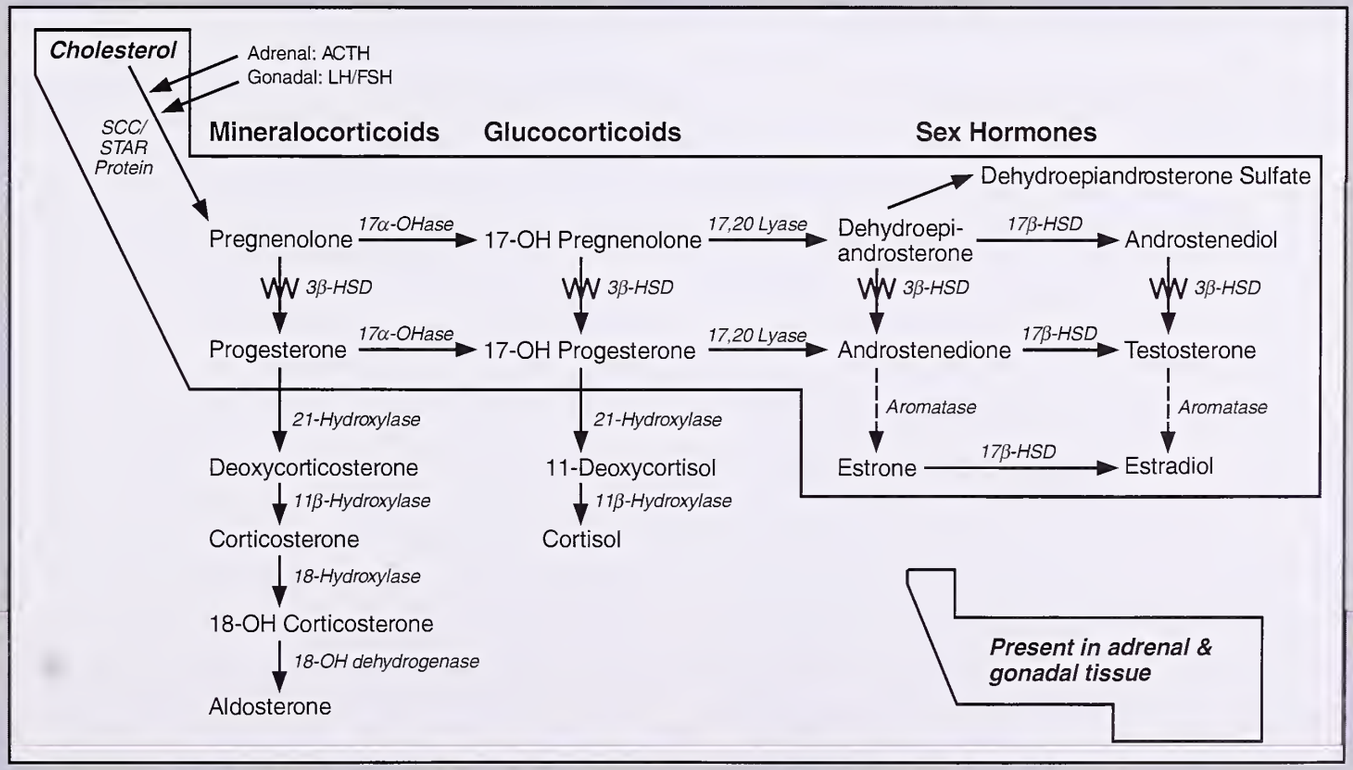
3β-Hydroxysteroid dehydrogenase/Δ5→Δ4 isomerase (3β-HSD) catalyzes the conversion of Δ5-3β-hydroxysteroid to Δ4-3β-ketosteroids in the adrenals, gonads, and in many extra-adrenal and extra-gonadal tissues (Figure 1).[1,2] The genetic control of 3β-HSD expression differs between the intra- and extra-adrenal and gonadal tissues.[3,4] Disorders involving decreased 3β-HSD activity in the adrenals and gonads have long been recognized. Recently, the discovery of 3β-HSD genes has led to the understanding of the molecular basis of 3β-HSD deficiency disorder. This review relates the clinical, biochemical, and molecular basis of severe, classic 3β-HSD deficiency disorder, and relates the molecular findings in patients exhibiting hormonal evidence of mildly decreased adrenal 3β-HSD activity. The latter led to the diagnosis in the last decade of mild, late-onset 3β-HSD deficiency disorder.

PATHOPHYSIOLOGY OF 3β-HSD DEFICIENCY

Classic 3β-HSD deficiency in humans occurs concomitantly in the adrenals and gonads, and is transmitted by an autosomal recessive trait.[3-5] Thus, regulation of adrenal/gonadal 3β-HSD activity in humans is under single-gene control. The enzyme deficiency in the adrenals results in cortisol deficiency, with or without aldosterone deficiency, and leads to increased corticotropin secretion and increased production of pregnenolone (Δ5-P), 17-hydroxypregnenolone (Δ5-17P), dehydroepiandrosterone (DHEA), and androstenediol (Figure 1).[3-8] Severe 3β-HSD deficiency produces congenital adrenal hyperplasia (CAH) with salt-wasting and ambiguous genitalia in male infants due to fetal testicular testosterone deficiency[5] and in female infants probably due to the effect of excess DHEA metabolites produced from the fetal adrenals.[3-7] Not all affected female infants have virilization.

The clinical spectrum of 3β-HSD deficiency at birth, however, **includes both salt-wasting and non–salt-wasting forms independent of the extent**

Figure 1
Schematic of Adrenal and Gonadal Steroidogenesis



Dotted arrow: major pathway in ovaries and minor pathway in testes and adrenal cortex.
FSH, follicle-stimulating hormone; LH, luteinizing hormone; SCC, cholesterol side chain cleavage; STAR, steroidogenic acute regulatory protein; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 17α-OHase, 17α-hydroxylase.

of genital ambiguity.[3,5,8] Non–salt-wasting severe 3β-HSD deficiency during childhood is associated with premature acne and/or premature sexual hair growth, and with growth acceleration in both sexes.[3,8] Clitoromegaly occurred in 1 affected girl.[3] During adolescence and adulthood, varying degrees of hypogonadism occur in males,[4,7,9] and hirsutism, irregular menses, and polycystic ovaries occur in females.[8,9] During the last decade, however, a concept of a so-called mild, late-onset 3β-HSD deficiency disorder was introduced and reported to occur in 3% to 60% of hirsute females,[10-15] and in 1.5% to 13% of premature pubarche children.[12,16] Diagnosis was based on Δ5-17P and DHEA levels >2 standard deviations (SD) above normal mean corticotropin-stimulated levels and on ratios of Δ5-17P:17-hydroxyprogesterone (17-OHP), Δ5-17P:cortisol, and/or DHEA:androstenedione (Δ4-A). Questions regarding the validity of the hormonal diagnostic criteria for mild 3β-HSD deficiency disorder remain, however, since no definite genetic evidence is available to document that such mild Δ5 steroid abnormalities to corticotropin stimulation result from mild variants of 3β-HSD deficiency CAH.[10-16]

Biochemically, 3β-HSD deficiency in both salt-wasting and non–salt-wasting patients is present only in the adrenals and gonads and not in the extra-adrenal/gonadal tissues. This indicates independent genetic regulation of the 3β-HSD enzyme between the intra- and extra-adrenal/gonadal tissues.[3,4] **The presence[4-6] or absence[3,6-8] of 3β-HSD deficiency in the aldosterone biosynthetic pathway in patients with severe 3β-HSD deficiency suggests varying genetic makeup among the forms of the disorder**.

THE GENETICS OF 3β-HSD GENES AND PROTEINS

In humans, 2 genes (type I and II) encoding 3β-HSD protein have recently been characterized.[17-20] Both genes are located in the chromosome 1p11-13 region, are 7.84 to 7.88 kb in length, and consist of 4 exons and 3 introns.[19,21] The type I and II 3β-HSD proteins are 93.5% homologous in amino acid sequence.[17-20] **Type I gene expression occurs primarily in the placenta, mammary gland, and skin. Type II gene expression occurs in the adrenals and gonads**. The 3β-HSD is membrane-bound in the endoplasmic reticulum and mitochondria.[22,23] The putative functional domains in both genes include 2 predicted membrane-spanning segments in exons III (codons 73 to 90) and IV (codons 286 to 305) and 2 suggested membrane-spanning segments in the 5' and middle regions of exon IV. In the type I gene, an additional membrane-spanning segment is suggested in exon II. These putative membrane-spanning domains are predicted to be critical gene sites.

In vitro kinetics of human type I and II 3β-HSD exhibited greater type I than type II 3β-HSD activity.[18,19,24] The Km values for Δ5-P, DHEA, and dihydrotestosterone of the type I 3β-HSD were approximately 5-, 6-, and 10-fold lower, respectively, than the Km values of the type II 3β-HSD. The maximum velocity (V_{max})/Km values for Δ5-P, DHEA, and dihydrotestosterone of the type I 3β-HSD were approximately 6-, 4-, and 3-fold greater, respectively, than the V_{max}/Km values of the type II 3β-HSD.[18,19,24] Thus, the type I enzyme should efficiently convert the predictably low Δ5 steroid concentrations in the peripheral tissues. 3β-HSD in the liver, kidney, lung, brain, and adipose in humans is not yet characterized. Additionally, three 3β-HSD pseudogenes or related genes have been identified by screening a human leukocyte genomic DNA library.[25] These pseudogenes, which contain stop codons and/or deletions in the coding regions, were suggested to have diverged from the type I gene millions of years ago.[25]

In other species, several types of 3β-HSD genes have been identified.[22,23] In rats and mice, the type I gene encodes for adrenal/gonadal 3β-HSD, the type II gene for liver 3β-HSD, the type III gene for liver and liver/kidney 3β-HSD, and the type IV gene for placental/skin and kidney 3β-HSD. The macaque type I gene encodes for gonadal 3β-HSD, and the bovine type I gene encodes for ovarian 3β-HSD. Thus, it is apparent that **the regulation of 3β-HSD expression in various tissue sites is under tissue-specific and independent genetic control in humans, rodents, and other mammalian species**.

MOLECULAR BASIS OF 3β-HSD DEFICIENCY CAH

The human type II 3β-HSD gene encodes specifically for adrenal/gonadal 3β-HSD enzymes. Thus, CAH resulting from 3β-HSD deficiency was predicted to result from deleterious mutations in the type II 3β-HSD gene. Analysis of the type II gene in all but 2 alleles from unrelated patients of various ethnic background with salt-wasting 3β-HSD deficiency revealed a premature stop codon or frameshift and subsequent premature stop codon in the gene due to a homozygous or compound heterozygous point mutation involving codons 171, 273, and 318, an insertion mutation between codons 186 and 187, or combined mutations at codons 248 and 249.[26-29] The resulting truncated 3β-HSD protein lacks 3β-HSD activity, causing the salt-wasting disorder. Two patients, however, had a missense mutation at codon 142 (Glu→Lys) or 253 (Thy→Asn) in 1 allele, and a 186/Ins C/187 mutation or W171X premature stop codon in the second allele.[30] These mutant 3β-HSD proteins translated by the cells transfected with the mutant genes via mutagenesis exhibited no enzyme activity in vitro (Table 1). The regions of codons 142 and 253 are well conserved throughout species, suggesting that these regions of amino acid residues are critical for 3β-HSD activity.[30]

Analysis of the type II 3β-HSD gene from patients with non–salt-wasting but severe 3β-HSD deficiency revealed primarily missense mutations in coding regions of the gene in all[30-35] but 2 alleles (Figure 2, page 8),[32, 34] including a point mutation involving codons 82 (Ala→Thr), 100 (Asn→Ser), 129 (Gly→Arg), 173 (Leu→Arg), 245 (Ala→Pro), and 254 (Thy→Asp).[31-35] In vitro, the apparent relatively specific efficiency to convert Δ5-P to progesterone (P) and DHEA to Δ4-A in monkey kidney transformed (COS) cells or homogenates transfected with the mutant genes by mutagenesis was compared with the 100% activity of wild-type 3β-HSD, revealing activities of 11.9% and 13.1%, respectively, by the codon 245 mutant protein (Ala→Pro); of 2.7% and 11% respectively by the codon 100 mutant protein (Asn→Ser); and of 2% and 4.7%, respectively, by the codon 129 mutant protein (Gly→Arg) (Table 1). These degrees of 3β-HSD activity were sufficient to prevent aldosterone deficiency, resulting in the non–salt-wasting disorder. The codon 254 (Thy→Asp) mutation exhibited no enzyme activity in vitro.[34] A second allele mutation in the type II gene, however, was not identified in this case to compare the phenotype with the genotype. The mutant 173 (Leu→Arg) and 82 (Ala→Thr) genes were not sufficiently studied to compare with the phenotype of the patients.[31,33] In non–salt-wasting sibs with the codon 129 missense mutation in 1 allele, the second allele had a G→A mutation in intron 3 at nucleotide 6651, 6 bases upstream from exon IV.[32] This may create a new splicing junction and affect the normal splicing of the mRNA. Type I gene sequences were normal in all alleles of patients with severe 3β-HSD deficiency who were examined.[27,28,32]

In general, review of the type II 3β-HSD gene mutations in 3β-HSD deficiency CAH indicates that **the salt-wasting form results from a homozygous or compound heterozygous mutation involving grossly altered 3β-HSD gene structures or alteration in amino acid residues in the conserved region of the gene. The non–salt-wasting form appears to result from amino acid substitution mutations** in the

Table 1
In Vitro 3β-HSD Activity of Missense Mutant Type II 3β-HSD Genes and Its Comparison to the Phenotype[30-35]

Mutant Gene Tested					Clinical Phenotype			
		V_{max}/Km						
Genotype	Protein Synthesis	Δ5-P→P	DHEA→Δ4-A	Second Allele Genotype	SW or NSW	Genetic Male With Ambiguous Genitalia	Genetic Female With Hirsutism and Menstrual Disorder	Premature Pubarche
Wild-type	+	100%	100%		–	–	–	–
Mutant gene:								
253 (Tyr→Asn)	+	0	0	186/Ins C/187 frameshift/ stop	SW	Yes	–	N/A
142 (Glu→Lys)	+	0	0	171 Trp→stop	SW	Yes	–	N/A
245 (Ala→Pro)	+	11.9%	13.1%	Homozygous	NSW	Yes	–	N/A
100 (Asn→Ser)	+	2.7%	11.0%	Homozygous	NSW	Yes	–	N/A
129 (Gly→Arg)	+(↓)	2.0%	4.7%	n6651 Intron 3 mutation	NSW NSW	Yes –	– Yes	Yes Yes
254 (Thy→Asp)	+	0	0	Not found(?)	NSW	–	Yes	No
173 (Leu→Arg)	Not done	Not done	Not done	Homozygous	NSW NSW	Yes –	– N/A	N/A N/A
82 (Ala→Thr)	Not reported	Not reported	Not reported	Homozygous	NSW NSW NSW NSW	Yes Yes – –	– – N/A No	Unknown No Yes No

V_{max}/Km, the first order rate constant is the index for apparent relative specific efficiency;
SW, salt-wasting; NSW, non–salt-wasting; N/A, not applicable due to young age

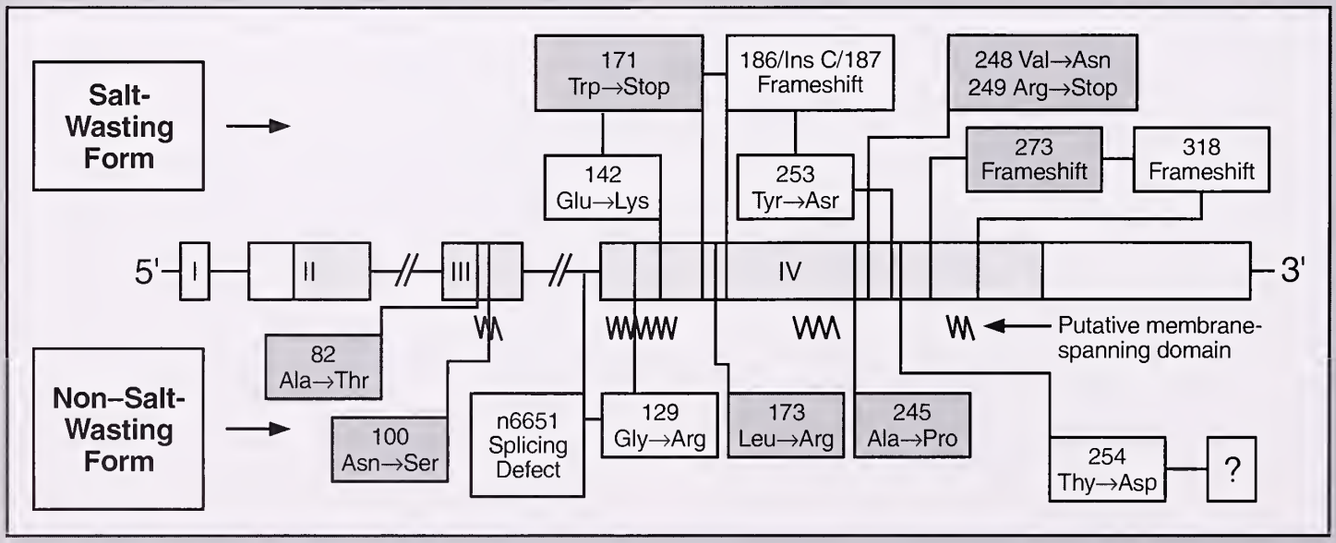
less conserved region of the gene in at least 1 allele. These **type II 3β-HSD gene findings in patients with classic 3β-HSD deficiency suggest that CAH with this enzyme deficiency results from type II 3β-HSD gene mutation**, and the phenotype correlates well with the genotype in classic 3β-HSD deficiency disorder.

TYPE II 3β-HSD GENE FINDINGS IN PATIENTS WITH HORMONAL EVIDENCE OF MILDLY TO MODERATELY DECREASED ADRENAL 3β-HSD ACTIVITY

During the past decade, the so-called mild, late-onset variant of 3β-HSD deficiency has been diagnosed in premature pubarche children[12,16] and in hirsute females, with or without menstrual disorders,[10-15] when corticotropin-stimulated Δ5-17P and DHEA levels and ratios of Δ5-17P:17-OHP, Δ5-17P: F, or DHEA:Δ4-A were >2 SD above mean values of pubertal stage-matched normal subjects. The hormonal criteria, however, have not been universally accepted for diagnosing the mild, late-onset disorder because the hormonal abnormalities were not outstanding compared with mild 21-hydroxylase deficiency.[36, 37] In mild 21-hydroxylase deficiency, corticotropin-stimulated 17-OHP levels were >21 SD above homozygous normal mean values, and >6 SD above mean levels of 21-hydroxylase deficiency carriers.[36,37] In addition, unusually large numbers of hirsute females and premature pubarche children had mild variants of 3β-HSD deficiency by the previous hormonal criteria.[10-16] Further, such hormonal diagnosis was not based on any genotypic proof. **Thus, the validity of the hormonal criteria published previously for diagnosing mild 3β-HSD deficiency disorder**, including this author's work, **is questionable.**

The mild variants of 3β-HSD deficiency producing CAH would likely result from a less deleterious mutation in the type II 3β-HSD gene. The type II 3β-HSD gene sequences—including regions of a putative promoter, all exons, and exon and intron boundaries—were normal in 5 premature pubarche children and 5 hirsute females whose corticotropin-stimulated levels were at 2.5 to 6.5 SD above the normal mean for 5-17P; 2.5 to 7 SD for DHEA; 2.5 to 4.3 SD for Δ5-17P:F ratio; and 3 to 8.6 SD for DHEA:Δ4-A ratio, indicating mild, nonclassic 3β-HSD deficiency by the previous hormonal criteria.[38] Further, corticotropin-stimulated hormonal profiles of 3 carrier mothers for severe 3β-HSD deficiency with a single allele mutation in the type II 3β-HSD gene were appropriately normal.[38] These findings suggest that, **hormonally, mildly or moderately decreased adrenal 3β-HSD activity is not caused by mild variants of 3β-HSD deficiency resulting from type II 3β-HSD gene mutations in 1 or both alleles.** Furthermore, **proven carriers of severe 3β-HSD deficiency do not appear to express decreased adrenal 3β-HSD activity**. Recently, Zerah et al[39] reported normal type I and II gene sequences in hirsute females with variably mildly decreased adrenal 3β-HSD activity as having nonclassic 3β-HSD deficiency.[39] **It is now apparent that children with premature pubarche and hirsute females with mildly decreased adrenal 3β-HSD activity and normal type II 3β-HSD gene sequences do not have the mild variant of 3β-HSD deficiency CAH**. The etiology of this mildly decreased adrenal 3β-HSD activity remains unknown.

Figure 2
Reported Mutations in the Type II 3β-HSD Gene in Patients With Severe (Classic) 3β-HSD Deficiency Congenital Adrenal Hyperplasia



☐ Regions of the gene
☐ Homozygous mutation reported[26-35]

CONCLUSIONS

Except for 1 allele of 1 patient, the patients with 3β-HSD deficiency disorder exhibiting unequivocal clinical and hormonal abnormality had type II 3β-HSD gene mutation in all alleles.[30-35] The patients exhibiting mildly to moderately decreased adrenal 3β-HSD activity, which led to the diagnosis of so-called mild, late-onset 3β-HSD deficiency disorder by the previously published hormonal criteria, had no mutation in the type II 3β-HSD gene in any allele.[38,39] Therefore, the previously published hormonal criteria recommended for diagnosing mild, late-onset 3β-HSD deficiency are not appropriate for diagnosing late-onset or nonclassic 3β-HSD deficiency in premature pubarche children or in females with hirsutism and/or menstrual disorders.

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Abstracts From the Literature

The Role of the *obese* Gene and Its Product in Obesity

Much attention is being given to the genetic predisposition to obesity. A review of several pertinent papers and the interrelationship of the data presented therein follows. Zhang et al[1] cloned the *obese (ob)* gene in mice and humans. A mutation found in the *ob/ob* mouse gene prompted studies of the biologic effects of its protein product (termed the OB protein, or leptin, a name derived from the Greek word leptos, or thin) in normal, heterozygous, and homozygous abnormal mice. The *ob* gene, sited on mouse chromosome 6, has 2,852 bases and codes for a 167 amino acid propeptide and a mature protein of 146 amino acids. Messenger RNA of the *ob* gene is expressed only in white adipose tissue of the mouse. Morbid obesity occurs in the C57BL/6J *ob/ob* mouse due to increased feeding and decreased activity. Type II diabetes mellitus and hyperinsulinemia also occur. A C→T transversion in codon 143 of the *ob* gene alters arginine to a stop codon (Arg143Stop), and prevents translation of this gene product.[1]

Utilizing recombinant mouse OB (rmOB) protein, Halaas et al[2] raised a rabbit polyclonal antibody, and by immunoprecipitation and SDS-PAGE gel electrophoresis demonstrated its presence in the plasma of wild-type C57BL/Ks *db/+* and C57BL/Ks *db/db* mice and in normal slim human subjects, but not in C57BL/6J *ob/ob* mice. (C57BL/Ks *db/db* mice resemble the *ob/ob* mice phenotypically, but as will be noted later are resistant to the effects of OB protein.) Halaas et al then administered rmOB protein (5 mg/kg intraperitoneally daily for 15 to 33 days) or phosphate-buffered saline (PBS) to female C57BL/6J *ob/ob* and C57BL/Ks *db/db* mice. The following findings were observed in the rmOB protein-treated C57BL/6J *ob/ob* mice: (1) significant weight loss within 4 days and a sustained weight loss reaching 40% of initial body weight by 33 days of treatment; (2) a 60% decrease in food intake; (3) a 95% decrease in body fat content in the C57BL/6J *ob/ob* mice; (4) a 50% decrease in plasma glucose concentration; (5) significantly greater weight loss than in pair-fed C57BL/6J *ob/ob* mice; and (6) equivalent weight and body fat reducing potencies of recombinant human OB and rmOB proteins in this model. No effect of rmOB protein was noted in C57BL/Ks *db/db* mice, indicating that this strain is resistant to the biologic effects of rmOB protein, perhaps due to an abnormality in the as yet unidentified receptor for rmOB protein. In wild-type female CBA/J +/+ mice receiving twice daily injections of rmOB protein (12.5 mg/kg/d), body weight decreased approximately 10% but fat mass declined 95%.

Pelleymounter et al[3] demonstrated in C57BL/6J *ob/ob* mice: (1) a dose-response relationship between weight loss and rmOB protein; (2) a decrease in food and water intake, serum glucose and insulin concentrations, and oxygen consumption in mice receiving rmOB protein; and (3) an increase in body temperature and locomotor activity. Campfield et al[4] reported that: (1) as weight increased, the effects of rmOB protein administered intraperitoneally in C57BL/6J *ob/ob* mice were reversible when the protein was withdrawn; (2) rmOB protein decreased food intake and weight of diet-induced obese mice; (3) acute intravenous injection of rmOB protein lowered food intake for more than 7 hours in C57BL/6J *ob/ob* mice; and

(4) injection of rmOB protein into the lateral ventricle of C57BL/6J *ob/ob* mice decreased food consumption for at least 7 hours.

1. Zhang Y, et al. *Nature* 1994;372:425-432.
2. Halaas JL, et al. *Science* 1995;269:543-546.
3. Pelleymounter MA, et al. *Science* 1995;269:540-543.
4. Campfield LA, et al. *Science* 1995;269:546-549.

***Editor's comment**: Data reported in these 3 articles[2-4] indicate that the OB protein (leptin): (1) circulates in mice and humans and is thus a hormone (and that fat is a gland of internal secretion and hence an endocrine gland); (2) induces weight loss and decline in body fat mass by both a decrease in caloric intake and an increase in activity; and (3) acts through a receptor, probably located within the central nervous system, and is active in obese syndromes associated not only with absence of this protein but also in diet-induced obesity. Establishing immunologic methods for the measurement of plasma levels of leptin; defining its normal physiologic role in the regulation of body composition, carbohydrate metabolism, the secretion of insulin, and other glucoregulatory hormones; elucidating its mechanisms of action; and exploring the legion of possible malfunctions of leptin production, action, or therapeutic applicability in a variety of diseases such as type II diabetes mellitus, polycystic ovary syndrome, and anorexia nervosa auger an exciting future for this peptide.*

Allen W. Root, MD

Gonadoblastoma: Molecular Definition of the Susceptibility Region on the Y Chromosome

It has been known for some time that gonadoblastomas are more common in Turner syndrome patients who have some of the Y chromosome still present. Gonadoblastomas are rare neoplasms composed of aggregates of germ cells mixed with smaller epithelial cells resembling mature Sertoli and granulosa cells. Gonadoblastomas arise within dysgenetic gonadal tissue of individuals who possess a Y chromosome or part of a Y chromosome. The authors of this paper used molecular markers from the Y chromosome to demonstrate the area of the Y chromosome that was missing in the individuals who developed gonadoblastomas, thus mapping the gonadoblastoma locus (*GBY*) on the Y chromosome. All individuals with gonadoblastomas had region 3 and region 4 of the Y chromosome present in their Y chromosomal material. All other regions of the Y chromosome were missing. Some of the study population were lacking the *SRY* gene, while others had it. Copies of 2 Y-linked gene families: *TSPY* (testis-specific protein, Y-encoded) and *YRRM* (Y-chromosome RNA recognition motif) were present in all patients. These 2 gene families have sequences dispersed over many regions of the Y chromosome. It seems likely that all the copies are not active; however, this is currently unproven. It is likely that most, if not all, patients with gonadoblastoma will have at least some copies of the genes present, despite large deletions of their Y chromosome.

The analysis of the DNA in this part of the Y chromosome is complicated because the interval lies in a region of XY homology and the PCR product from both the Y and X chromosomes are almost the same size. The authors estimate that the GBY critical region is about 1 to 2 megabases. Copies of the *TSPY* gene but not the *YRRM* gene fall within the GBY critical region according to the deletion mapping.

Two tumors were sampled that showed expression of both *TSPY* and *YRRM* genes. Interestingly, in one patient with unilateral gonadoblastoma, the contralateral unaffected streaked gonad did not show expression of either gene.

These studies have not directly implicated either gene in the etiology of gonadoblastoma. However, they certainly raise the issue of whether there is only one gene or a critical region or possibly multiple loci on the Y chromosome involved in the production of gonadoblastoma.

Tsuchiya K, et al. *Am J Hum Genet* 1995;57:1400-1407.

***Editor's comment**: Pediatric endocrinologists have wondered for some time why individuals with Turner syndrome are at risk for gonadoblastoma. It was suggested in the 1980s that only those individuals with Turner syndrome who had Y chromosome material still present in the gonadal streak were at risk. The present study isolates the specific area of Y chromosome (regions 3 and 4) that puts these individuals at risk. Thus, the gonadoblastoma region of the Y chromosome has been identified and gonadoblastoma tissue shows expression of at least 2 genes not expressed in nongonadoblastoma tissue.*

TSPY *falls within the critical region and has 30% identity and 56% similarity with the* SET *protein. The* SET *gene has been implicated in acute undifferentiated leukemia and thus may play some kind of role in tumorigenesis.*

A great deal more will have to be learned regarding the presence of multiple copies of genes on the Y chromosome. It is not clear whether there are individual or polymorphic variations in copy number or variations in these Y sequences. More needs to be known about the expression of alternate transcripts and the presence of the untranscribed control elements of these genes. Nevertheless, the significance of this study for Turner syndrome patients is that it shows that deletions of the Y chromosome that leave regions 3 and 4 intact put these individuals at risk for gonadoblastoma.

Judith G. Hall, MD

Microphallus: Eventual Phallic Size Is Dependent on the Timing of Androgen Administration

Husmann and Cain produced 2 animal (rat) models of hypogonadotropic hypogonadism in utero. Persistent microphallus and sexual infantilism occurred. Treatment with dihydrotestosterone (DHT) in large doses was begun at 7, 28, 56, and 84 days of age. To evaluate the effect of treatment, the length (stretched) and weight (autopsied) of the penis was measured. The androgen receptor protein found in the penile corpora, which is necessary for penile growth and which disappears as the penis reaches end-stage growth, was measured immunohistochemically.

Early exposure to androgens (before 56 days) resulted in diminutive penile growth, apparently due to accelerated downregulation of the androgen receptor. Although late administration of androgen enhanced penile length significantly (Figure 1), penile weights (reflecting penile widths) remained subnormal (<2.5 standard deviations [SD] below the normal mean).

The authors believe, based on current clinical and experimental data, that brief androgen therapy of the neonate with micropenis is necessary to determine if the phallus will respond; this is necessary in considering the sex of rearing. Interval therapy during childhood is not recommended. Treatment with androgens to stimulate maximal phallic growth should be initiated when the child is >12 years of age. The statement is made that evaluation of the adult population with a history of micropenis reveals that interval androgen therapy during childhood does not result in any significant size advantage of the penis compared with that of the untreated child. Unfortunately, delaying pharmacologic therapy does not result in complete development of phallic growth (weight, therefore width). Further studies reportedly are underway using the 2 rat models.

Husmann DA, Cain MP. *J Urol* 1994;152:734-739.

Editor's comment: *Although published in 1994, this article only recently was called to my attention (by Dr. Dan Metzger of the University of British Columbia, and British Columbia Children's Hospital, Vancouver). A subsequent abstract was presented at the American Academy of Pediatrics meeting in April 1995 dealing with analogous studies in humans (Cain et al. "Micropenis Secondary to Hypogonadotropic Hypogonadism: Clinical Evaluation of Early Versus Late Hormonal Therapy").*

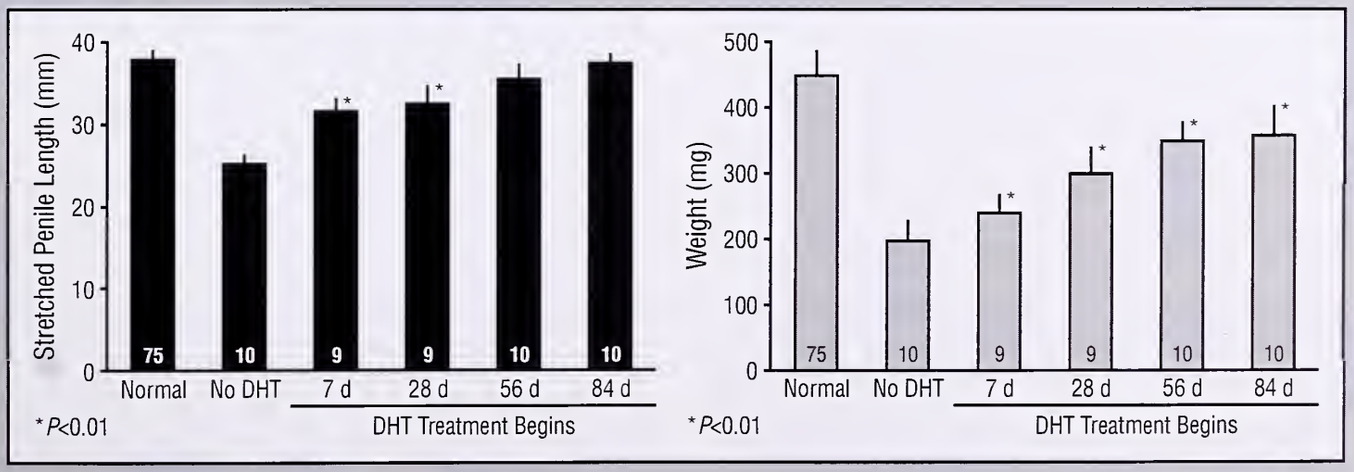
Twenty-five patients met the criteria of micropenis, ie, stretched penile length at diagnosis <2.5 SD below the mean and laboratory criteria consistent with gonadotropin hormone-releasing hormone deficiency. Early hormonal therapy was defined as >20,000 IU of human chorionic gonadotropin (HCG), <6 months treatment with testosterone cream, or combined HCG and testosterone treatment >3 months, by 7 years of age. Delayed treatment was defined as initiation of treatment after 11 years of age. Ten and 15 patients were categorized into each group, respectively.

The median age at diagnosis in the early treatment group was 4 years (range, birth to > 7 years). Final evaluation was at 20.5 years (16 to 28 years). All continued to have micropenis (<-2.5 SD). Fifteen received late treatment (median age, 15 years). Final evaluation was at 21.3 years (15 to 37 years). Stretched penile length was within normal range in 13 of the 15 patients treated late. The authors concluded that the clinical data support the following hypothesis: Improved phallic growth occurs with delayed hormonal therapy for micropenis secondary to hypogonadotropic hypogonadism.

These data from humans and the conclusions drawn are intriguing because of the similarity to the conclusions reached from studying the animal model.

Is the parallelism justified? My answer is: Possibly, but only possibly. The studies are important because of the attempt to study biochemical and anatomic science with treatment in the rat, and make clinical observations to support or reject the hypotheses derived from the animal studies. Congratulations are extended to the authors for their approach and efforts. However, questions to be raised include: (1) Were the phalluses

Figure 1



Penile length and weight are given for hypogonadotropic rats in relation to when dihydrotestosterone (DHT) treatment was begun. DHT treatment was effective in increasing length in all, but the increase was greater in rats treated late.

in the older age group relatively larger before treatment? The median age at diagnosis was 12 years, in contrast to 4.0 years for the early treatment group. (2) Were the widths of the phalluses greater in the late treatment group—before and/or after? Widths of the flaccid phallus could have been measured in the human, although this was not possible in the rat. (3) Were any of the children in the early treatment group deficient in growth hormone (GH)? GH deficiency is associated with micropenis, and particularly so when luteinizing hormone and GH are both deficient.

Regardless of the answers to these questions, extended delay of treatment (usually with androgens) for individuals with micropenis is advisable. However, in my experience there are some young children who have significant psychological consequences as a result of not being able to bare themselves in the dressing room and/or stand to urinate with their peers. These children should be treated early, in my opinion, if they are being raised as males. Emphasis should be made in respect to interpreting these data that the subjects all allegedly had micropenis secondary to hypogonadotropic hypogonadism, and the observation and deductions should not be construed outside micropenis of this origin.

Robert M. Blizzard, MD

The Role of the Sulfonylurea Receptor in Insulin Secretion

Review of several articles permits the following deductions. In response to glucose, there is depolarization of pancreatic β cells, transient increase in cytoplasmic levels of CA^{++}, and release of insulin. These processes are regulated by adenosine triphosphate (ATP)-sensitive K^+ channels that are blocked by glucose-induced increase in the cytosolic ratio of ATP:adenosine diphosphate (ADP), thus resulting in membrane depolarization and increased release of stored intracellular CA^{++}.[1] Sulfonylureas stimulate insulin secretion by blocking ATP-sensitive K^+ channels, thus depolarizing pancreatic β cells. Aguilar-Bryan et al[2] have identified the genes for the endogenous sulfonylurea receptors (SURs) of the rat and hamster; they code for 1,582 amino acid proteins with a molecular weight of 177 kd and 13 transmembrane domains that share homology with the cystic fibrosis transconductance regulator (CFTR) and P-glycoprotein multidrug resistance (MDR) genes, both membrane transport proteins. The SUR is associated with but is not the K^+ receptor; it may regulate activity of this monovalent cation channel by affecting the phosphorylation of the K^+ channel or by sensing the ATP:ADP ratio.

In humans, the gene for the SUR is localized to chromosome 11p15.1, the same chromosomal location to which persistent hyperinsulinemic hypoglycemia of infancy (PHHI) has been linked. In patients with PHHI, an autosomal recessive disease of severe hypoglycemia and unregulated insulin secretion, Thomas et al[3] demonstrated that the gene for the human SUR was abnormal. In 13 families, a homozygous guanine to adenine (G→A) mutation was present in the region of the gene coding for the second nucleotide binding fold (a portion of the protein that interacts with cytosolic nucleotides), leading to an abnormal frameshift and inclusion of a stop codon, thus resulting in a truncated protein. In one family a G→A mutation in a codon preceding the exon coding for the second nucleotide binding fold region resulted in abnormal splice sites within this important region. These studies demonstrate the importance of the endogenous SUR in the regulation of insulin secretion. Inactivation of this receptor results in unregulated insulin secretion, implying that normally this receptor inhibits insulin release by maintaining the activity of the ATP-dependent K^+ channels within the pancreatic β cell.

1. Philipson LH, Steiner DF. *Science* 1995;268:372-373.
2. Aguilar-Bryan L, et al. *Science* 1995;268:423-426.
3. Thomas PM, et al. *Science* 1995;268:426-429.

***Editor's comment**: These articles illustrate the principle that many therapeutic agents reflect the action of endogenous substances as yet undiscovered. Thus, identification of the SUR implies the presence of an endogenous ligand for this receptor that must be involved in the regulation of insulin secretion and carbohydrate metabolism. One wonders about the chemical composition and source of this endogenous ligand and whether the endogenous ligand and/or its receptor might be aberrant not only in subjects with PHHI but also in patients with other disorders of energy homeostasis, perhaps with islet cell tumors or some forms of obesity.*

Allen W. Root, MD

Lymphocytic Hypophysitis: Clinicopathological Findings

A clinicopathologic description of 16 (2 male and 14 female) patients with lymphocytic hypophysitis was presented. In 10 of the 14 female cases, the presentation was associated with pregnancy (2 in the second trimester, 2 in the third trimester, and 6 postpartum). Clinical presentations were diverse: 9 patients (56%) exhibited signs of expanding pituitary mass; 10 (63%) showed anterior pituitary hypofunction; 3 (19%) had diabetes insipidus; and 6 (38%) displayed hyperprolactinemia (4 associated with pregnancy and 2 attributable to a stalk effect). Three (19%) died due to progressive unrecognized hypopituitarism. In 1 patient (6%), elevated growth hormone (GH) levels with a resultant increase in insulin-like growth factor 1 were demonstrated. In 4 patients (25%), autoimmune thyroiditis was found. In 10 patients (63%) a pituitary mass mimicking an adenoma on computed tomography scans or magnetic resonance images was demonstrated, with 8 showing evidence of suprasellar extension. Antipituitary antibody testing was performed in 2 patients and yielded negative results. The

diagnosis of hypophysitis was made by pathologic studies in all patients. Light microscopy revealed lymphoplasmacytic infiltrate accompanied by varied numbers of neutrophils, eosinophils, and macrophages. Preserved cells were grouped in small islands surrounded by inflammatory infiltrate or fibrous tissue. Immunocytochemistry performed in 14 cases revealed the presence of GH and prolactin in all but 1 patient. There was absence of corticotropin immunoreactivity in 5 patients; of these, 3 had adrenal insufficiency and 1 was receiving treatment with steroids. In addition to confirming the findings of light microscopy, electron microscopy (8 patients) identified lactotroph cell hyperplasia or hyperactivity in 3 patients (1 male and 2 females, pregnancy related). In the 3 postmortem examinations, gross pituitary atrophy along with adrenal atrophy (presumably secondary to pituitary-target organ dysfunction) was found. The authors concluded that lymphocytic hypophysitis should be considered in the differential diagnosis of females presenting with pituitary enlargement in the peripartum period, in patients presenting with GH deficiency or excess associated with autoimmune disorders, and in patients presenting with rapidly enlarging pituitary masses with or without pituitary hormone dysfunction.

Thodou E, et al. *J Clin Endocrinol Metab* 1995;80:2302-2311.

***Editor's comment**: This is an important compilation of patients with lymphocytic hypophysitis that showed the great diversity and heterogeneity of the clinical picture of this disorder. The diagnosis of lymphocytic hypophysitis was confirmed by biopsy in all instances. The authors advocate conservative treatment on the basis of clinical suspicion to avoid aggressive surgical intervention. However, it was the experience of the authors, as well as of others, that there is no way to elucidate the final diagnosis before surgery. Antipituitary antibody testing in 2 patients produced negative results. Thus, the validity of these measurements to diagnose lymphocytic hypophysitis cannot be relied upon for diagnostic purposes. This group of patients with hypophysitis did not include children with hypopituitarism; therefore, it is hard to ascertain whether autoimmune hypophysitis occurs in children diagnosed with GH deficiency who do not exhibit pituitary masses on imaging studies and in whom the diagnosis of idiopathic GH deficiency is made. Most of the literature of lymphocytic hypophysitis relates to middle-aged patients, with a higher prevalence of females than males.*

Fima Lifshitz, MD

Final Height and Predicted Height in Boys With Untreated Constitutional Growth Delay

The authors reexamined 49 males at a mean chronologic age of 22.9 years (range, 20.4 to 31.2 years) who presented to their clinic at a mean age of 13.3 years (range, 7.3 to 16.4 years) and were diagnosed with constitutional delay of growth (CDG). The reexamination included measurements of standing height, using a Harpenden stadiometer, and testicular volume. At initial presentation, the diagnosis of CDG was made by documenting a standing height <5th percentile for chronologic age and a bone age retarded by 1 year or more in a boy who was born at term and had a birth weight of 2,500 g. Seventy-five percent of the boys had a history of late maturing parents. Heights of both parents were recorded. No patient with dysmorphic features, systemic disease, nutritional disorders, or suspected hormone deficiency was included in the sample. None of these men had received any chronic medical treatment, including anabolic steroids, during the intervening years. At the initial visit, the bone ages were determined by the methods of Greulich and Pyle and of Tanner-Whitehouse Mark II (TW2). Height predictions were calculated by the Bayley-Pinneau, TW2, and Roche-Wainer-Thissen (RWT) methods. Target height (TH) was defined as midparental height with 6.5 cm added and with 1 standard deviation (SD) defined as 4.25 cm. Paired *t*-tests and linear analyses were used for comparisons.

At the reexamination, the measured final height of these men was within the lower range of normal for the population, but significantly below their THs (by an average of 1.7 cm). There was a good correlation between the final height SD score (SDS), the initial bone age deficit, and the initial height SDS for bone age. No endocrine disorders became evident in these men and the mean testicular volume on reexamination was 19.0 mL (range, 10.3 to 25 mL). Predicted height by the Bayley-Pinneau method did not differ from the mean final height, and predictions by all 3 methods and by TH were significantly positively correlated with final height. Height predictions were not more accurate in boys with advanced versus younger chronologic age at initial presentation.

Sperlich M, et al. *Eur J Pediatr* 1995;154:627-632.

***Editor's comment**: This study provides important information for the pediatric endocrinologist counseling boys with CDG and their families. The authors have demonstrated that there is a good correlation between predicted heights, THs, and final heights. However, in untreated men with CDG the final heights are significantly lower than the THs. Interestingly, both the final heights and the THs were within the lower range of the population norm, suggesting a component of familial short stature present in these men. Their data suggest that final height in untreated CDG patients may be compromised. A table in the article summarizes similar findings from 10 other studies of final height in untreated men with CDG. All but one demonstrate a final height significantly lower than the TH.*

William L. Clarke, MD

***2nd Editor's comment:** In* GGH, *Vol. 11, No. 4, page 2, an article entitled "Predictive Factors in the Determination of Final Height in Boys With CDGP" was abstracted. The ultimate heights recorded in this article were less than expected for midparental height. You may wish to review the data abstracted from the article and the editorial comments by 2 of our editors in* GGH *11:4.*

Robert M. Blizzard, MD

One Gene, Three Chondrodysplasias: Déjà vu

It was recently shown that mutations of the *FGFR3* gene cause achondroplasia, hypochondroplasia, and thanatophoric dysplasia. This came as a surprise for some because the severity of the clinical phenotypes varies so much. Now it seems that the same phenomenon occurs with mutations of another gene, the so-called diastrophic dysplasia sulfate transporter (*DTDST*) gene. *DTDST* mutations have been detected in 3 autosomal recessive chondrodysplasias: diastrophic dysplasia (DTD), atelosteogenesis type II (AOII), and achondrogenesis type 1B (ACG-1B). The latter 2 conditions are lethal in the perinatal period. Both exhibit poor skeletal development; however, the defect is more severe in ACG-1B.

The *DTDST* gene was discovered in 1994 when mutations were found in patients with DTD.[1] As its name implies, the gene product acts to transport sulfate ions into cells. Although *DTDST* expression is widespread, the consequences of *DTDST* mutations are restricted mainly to cartilage, presumably because the proteins (proteoglycans) that occupy cartilage matrix are so highly sulfated. Regarding bone growth, it was suggested that defective sulfate uptake by chondrocytes leads to deficient sulfation of cartilage proteoglycans, which causes cartilage to function poorly as a template for endochondral bone growth.

Because qualitative similarities in skeletal radiographs and growth plate histology to DTD were observed, studies were carried out in both AOII and ACG-1B, which eventually led to finding mutations of *DTDST* in both recessive conditions. Five different mutations were detected in the 6 *DTDST* alleles from 3 patients with AOII, and 7 mutations were found in the 12 *DTDST* alleles from 6 patients with ACG-1B. Most interesting was that some of the same mutations were identified in the different conditions.

Thus, the 3 disorders are not only allelic, but they share common mutant alleles in different combinations. In other words, certain combinations of mutations appear to produce the clinical manifestations of DTD, other combinations result in OAII, while other combinations cause ACG-1B.

As addressed in both recent papers,[2,3] the simplest explanation for the findings is that all 3 disorders result from a common pathogenesis, which involves defective sulfate uptake by chondrocytes. The degree to which uptake is disturbed, which reflects how well the combined products of the 2 alleles function to transport sulfate, determines the clinical phenotype. The 3 disorders thus constitute what is often called a phenotypic series of disorders.

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***Editor's comment**: The number of chondrodysplasia gene loci seem to be shrinking. Indeed, if one considers the disorders that map to the* COL2A1 *(type II collagen) locus, which include the various spondyloepiphyseal dysplasias, Kniest dysplasia, Stickler dysplasia, hypochondrogenesis, and achondrogenesis type II, and to the* FGFR3 *and* DTDST *loci as discussed here, one can account for a very large percentage of all patients with chondrodysplasias. It will be interesting to see if this trend continues or if the number of chondrodysplasias associated with mutations at these loci have reached their limit.*

The revelations regarding the FGFR3 *and* DTDST *mutations bring up the issue of where, ie, what tissues, genes are expressed versus where disease manifestations arise when the genes are mutated. It is often true that they are the same. For example, mutations of type I and II collagen genes in osteogenesis imperfecta and spondyloepiphyseal dysplasias respectively produce manifestations in most tissues where the genes are expressed. In contrast, both* FGFR3 *and* DTDST *genes are expressed in many tissues, yet the pathologic consequences of mutations are restricted mainly to cartilage, especially the growth plate. For* DTDST, *this observation apparently reflects the much greater need for sulfate in cartilage compared with other tissues. For* FGFR3, *the explanation is not yet evident.*

William A. Horton, MD

Mutations of the Growth Hormone Receptor in Children With Idiopathic Short Stature

The authors studied 14 children with idiopathic short stature who had normal growth hormone (GH) secretion but low serum concentrations of GH-binding protein. They thought it likely that these patients had abnormalities in the gene for the GH receptor. They hypothesized that the mild form of insensitivity to GH could be caused by a mildly disruptive mutation of the gene for the GH receptor as compared with children with complete GH insensitivity such as Laron dwarfism. Four of the 14 children had PCR fragments that had altered migration mobility. Sequencing of the genes showed that 3 patients had a single mutation, while the fourth patient was a compound heterozygote. All had changes in the DNA that were confined to the extracellular domain of the receptor. It seems possible that the other 10 patients also had changes in the GH receptor gene that could not be picked up by mobility changes.

The implications are that heterozygote mutations of the GH receptor gene can have mild or severe growth consequences, depending on what the other gene is like. The patient who was a compound heterozygote was more severely affected than either of his heterozygote parents. Another child was more severely affected than his heterozygote mother, suggesting that his father might also carry an as yet undefined mutation.

These patients only had a marginal response to GH therapy, so appropriate therapy is unclear at this time.

Goddard AD, et al. *N Engl J Med* 1995;333:1093-1098.

***Editor's comment**: Good clinical criteria exist to suspect that an individual may have a problem with the GH-binding protein. These include mild to moderate short stature; the presence of normal GH levels but low serum concentration of GH-binding protein; and poor response to GH therapy. The mutations of the GH-binding protein gene that have been described are all*

in the extracellular domain of the protein. It can be anticipated that there will also be intracellular mutations.

This type of problem would be expected to run in families, with interaction between the 2 genes in the individual since the functional protein is a dimer. Further family studies are needed. The biology of GH and its receptor is being revealed through this type of molecular study of the experiments of nature.

For GH to stimulate release of insulin-like growth factor (IGF), the GH-binding sites must form a proper complex and produce an intracellular signal to activate the secretion of IGF-1. The classic form of GH receptor deficiency is Laron dwarfism, wherein the receptor is absent. Several hundred cases of classic Laron dwarfism have been identified; those patients with partial deficiency are just beginning to be described. Previously described heterozygotes for Laron dwarfism have been normal. Perhaps having an abnormal gene product is more disruptive than lacking a gene product from 1 of the 2 genes.

It is not at all clear what the optimal therapy for these patients will be. Perhaps if IGF-1 therapy is successful, it can bypass the GH receptor gene abnormality.

Judith G. Hall, MD

***2nd Editor's comment**: A related article was reviewed in the immediately previous issue of* GGH *(Vol 11:4) entitled "Evidence for Partial GH Insensitivity Among Patients With Idiopathic Short Stature" (*J Pediatr *1995;127:244-250). You as a reader may wish to reread the abstract and Dr. Lifshitz's editorial comment.*

Robert M. Blizzard, MD

Letters to the Editor

I would like to comment about an abstract published in the June 1995 (Vol. 11, No. 2) issue of *GROWTH, Genetics, & Hormones (GGH)*, "Identical Mutations in the *FGFR2* Gene Cause Both Pfeiffer and Crouzon Syndromes Phenotypes."

In 1981, DeNegrotti and I described a girl with Pfeiffer syndrome; her mother presented with a very mild expression of the disease but only at the level of cranium and face. Her hands and feet were normal.

One of the references in that report was a paper from Jackson et al that described a large Amish kindred with several individuals affected by different types of acrocephalosyndactyly, showing a great intrafamilial phenotypic variability. Our hypothesis was then that most of the autosomal dominant syndromes of acrocephalosyndactyly were the result of defects in just one gene, rather than the result of mutations in different genes. At that time, molecular studies were not yet available.

The abstract published in *GGH* seems to confirm our 14-year-old hypothesis, which was based only on clinical observations.

Sincerely,

Dr. José María Sánchez
Genetica Periconcepcional Y
Pediatrica Ecodiagnostico
Francisco Acuna De Figueroa 731
Buenos Aires, Argentina

Sánchez JM, et al. *J Med Genet* 1981;18:73-75.
Jackson CE, et al. *J Pediatr* 1976;88:963-968.

William L. Clarke, MD, reviewed the paper of Baron et al (*GGH* 1995;11[1]:6-7) on catch-up growth in rabbits after infusion of glucocorticoid into the tibial growth plate. Clarke states, "These data suggest that catch-up growth is intrinsic to the growth plate and not the result of a systemic hormonal mechanism." This seems to be the classic error in logic, ie, generalizing from the particular. It is analogous to the blind man deciding on the elephant's shape on the basis of feeling a single part. Baron and colleagues tend to do the same thing—but not quite. In fact, the experiment of Baron et al only shows what happens when glucocorticoids hit chondrocytes. Their experiment does confirm work extending over the past 50 years showing that excessive glucocorticoids are poison to the growth zone.

Best regards and congratulations on guiding *GGH* for a decade.

Sincerely yours,

H. David Mosier, MD
Department of Pediatrics
California College of Medicine
University of California, Irvine
Irvine, California

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Response to *Letter to the Editor*

Dear Dr. Blizzard:

Thank you for giving me the opportunity to respond to Dr. Mosier's letter. My reply follows:

The prevailing explanation for catch-up growth involves a central nervous system mechanism that compares actual body size to an age-appropriate set point and adjusts growth rate accordingly.[1] In contrast, we hypothesized that the mechanism governing catch-up growth resides not in the central nervous system but rather in the growth plate. To test this hypothesis, we asked whether transient suppression of growth by excess glucocorticoid within a single growth plate would lead to local catch-up growth. We administered dexamethasone directly into the proximal tibial growth plate of 6-week-old rabbits into the contralateral growth plate.[2] Dexamethasone slowed the proximal tibial growth rate during the 4-week infusion compared with the contralateral vehicle-treated control. After the infusion ended, the growth rate of the dexamethasone-treated side not only normalized but actually surpassed that of the control side, thus correcting approximately half of the growth deficit. This catch-up growth was observed solely in the growth plate in which the growth inhibition had occurred; growth in the distal tibia and in the femur was unaffected.

Since the catch-up growth was unilateral, it could not be explained by the prevailing neuroendocrine hypothesis. A neuroendocrine mechanism, or any systemic mechanism that involved circulating factors, would have affected all growth plates and thus could not by itself account for the observed anatomic specificity. Thus, the data suggest that the underlying mechanism is intrinsic to the growth plate.

In his letter, Dr. Mosier seems to be suggesting metaphorically that different mechanisms might contribute to catch-up growth under different conditions and the observed local mechanism might be just one component of catch-up growth. That hypothesis is quite plausible. In fact, in our published report we noted, "Our data do not exclude the possibility that both local and systemic mechanisms may contribute to catch-up growth under other circumstances."[2] As Dr. William Clarke commented in his review of our study, it would be interesting to see similar studies performed in models of other disorders associated with decreased growth velocity and subsequent catch-up growth.[3]

Sincerely,

Jeffrey Baron, MD
Senior Clinical Investigator
Developmental Endocrinology Branch
National Institute of Child Health and
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National Institutes of Health
Bethesda, Maryland

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Osteoporotic Syndromes in Childhood

Joseph M. Gertner, MD
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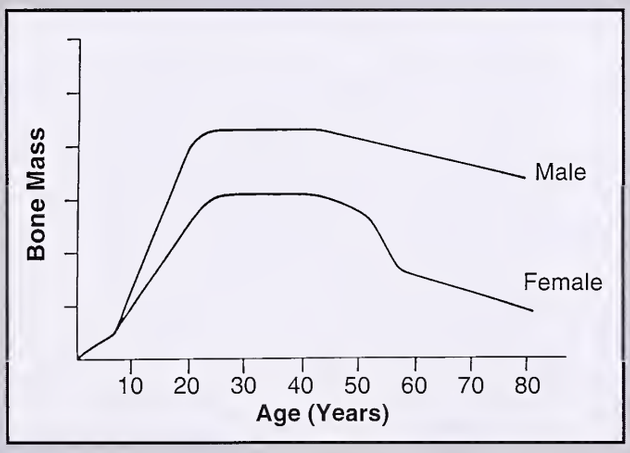
The structural framework of the body is the skeleton. The elements of the skeleton, which are fiber, matrix substance, and mineral, coexist in a highly regulated manner in this fascinating and complex system. Many skeletal components have extraskeletal counterparts, and the pathology within the skeleton may parallel extraskeletal disease. Interactions between the skeleton and other organs can cause extraskeletal dysfunction and vice versa.

Osteoporosis exists when the matrix and mineral within the bone are pathologically diminished. **Osteomalacia** exists when there is failure of mineralization despite the presence of adequate matrix. **Osteopenia** describes an abnormally low density of skeleton; however, it is insufficiently severe to cause symptoms or loss of function.[1] It differs from osteoporosis only in degree, and the terms are used interchangeably here.

Osteoporosis usually is regarded as a disease of later life; it occurs particularly in women and is associated with the hormone changes of menopause. The main emphasis in this review is on osteoporosis occurring in children. The causes, presentation, and treatment of metabolic bone disease in the newborn will be covered in a subsequent issue.

Figure 1



Diagrammatic representation of changes in bone mass (indicated in arbitrary units on the Y-axis) with age. Note the rapid gain of bone mass during childhood and adolescence, the subsequent slow decline until middle age and the rapid bone loss in women between 50 and 60 years of age (modified from Cooper[19]).

SKELETAL TURNOVER AND THE DEVELOPMENT OF OSTEOPOROSIS

Bone arises largely from cartilaginous derivatives of the mesodermal anlage. Both cortical and trabecular bone exist in a state of constant dynamic activity. **Osteoblasts** form new matrix and promote calcification of that matrix. **Osteoclasts** remove existing bone. Bone cells regulate each other's activity in a paracrine fashion, indicating **that the processes of bone formation and resorption are coupled.** During childhood a net gain of skeletal material occurs. A plateau is reached in the early 20s, followed by a decline (Figure 1). Bone **mineral** accretion in childhood results in somatic and skeletal growth, and the bones increase in density simultaneously, ie, mass of calcified tissue per unit volume of bone. Adult osteoporosis results primarily from bone resorption. In childhood, a similar imbalance may give rise to osteoporosis, but **failure of new bone to develop as the skeleton grows may reduce bone density by a mechanism unique to childhood.**

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In Future Issues

The Status of and Indications for Leg-Lengthening Procedures
Deborah Stanitski, MD

G Protein-Related Diseases
Andrew Shenker, MD

The Safety and Effectiveness of hGH Using Pharmacologic Dosing
Arnold Slyper, MD, PhD

Growth Hormone Treatment of Chronic Renal Failure
Richard Fine, MD

Gene Mapping and the Molecular Biology of Endocrine Disorders
Victor McKusick, MD

And Others

MEASUREMENT OF BONE DENSITY

Osteoporosis may be diagnosed from symptoms resulting from the collapse of a vertebral body or peripheral fracture. However, **the presence or absence of fracture is only a crude measure of the skeleton's integrity.** Thus, **extreme** degrees of osteopenia now can be diagnosed utilizing densitometric methods. In recent years these methods have become quite sophisticated. **Normative data for children are now available because of the low doses of radiation required.**[2] Both dual-energy densitometry (DXA) and quantitative computed tomography (qCT) provide usable data. The former is the most widely used method. The drawback is that the method measures the attenuation of an X-ray beam across a projected cross-section of bone (Figure 2). Therefore, the dimensions of the bone as well as the actual density of the skeletal mineral are recorded. The changes in these dimensions in childhood require that corrections based on the child's height and weight be applied.[3]

True bone density, ie, mass/unit volume, is measured by qCT. Both cost and radiation dose limit its use in children and available normative data are limited. The future development of low-dose radiation methods for qCT will prove beneficial.

SYMPTOMATOLOGY OF CHILDHOOD OSTEOPOROSIS

Osteoporosis in the elderly has been called the silent epidemic, an allusion to the unobtrusive way in which osteoporosis, usually painless until fractures occur, develops. The prime exception is osteopenia due to malignant infiltration of bone. **Osteoporosis in childhood is usually discovered by radiologic examination of at-risk children, after a fracture has occurred, or as a chance finding on X-ray films. In fractures of the lower limbs, it may be difficult to determine the existence of osteopenia.** In contrast, vertebral fractures in children are almost invariably due to local or generalized osteoporosis, unless there has been severe trauma. Vertebral fractures cause pain, deformity (kyphosis), and loss of height in the upper body segment.

Classification of childhood osteoporotic conditions is presented in Table 1. Detailed discussions of some of the individual causes then follow.

CLASSIFICATION OF CHILDHOOD OSTEOPOROTIC CONDITIONS

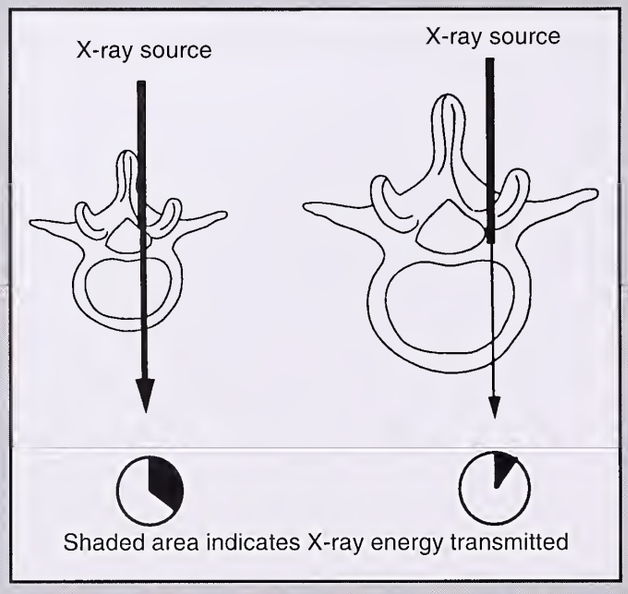
Genetic Defects

The bone matrix consists of collagen and a large number of noncollagenous proteins. **Genetic or acquired defects in the structure and/or assembly of collagen can lead to osteopenia, as in osteogenesis imperfecta (OI).**

Osteogenesis Imperfecta

This deforming bone disease is caused by heritable quantitative or qualitative disorders of type 1 collagen, the major collagen in bone.[4] Many cases of OI previously classified as recessive are now attributed to dominant germline mutations in a parent.

Figure 2



The diminished attenuation of a transmitted X-ray beam by a smaller bone means that body size must be taken into account in the interpretation of DXA "bone mineral density" readings.

Table 1
Classification of Osteoporotic Conditions of Childhood

Class	Disorder	Etiology
Genetic Defects of Matrix	Osteogenesis imperfecta	Mutations in one of type 1 collagen genes
	Homocystinuria	Cystathionine synthetase deficiency
	Menkes's syndrome	Mutation in copper transporting ATPase α polypeptide
Hormonal	Hypogonadism	
	Glucocorticoid excess	
	Thyrotoxicosis	
	Hyperparathyroidism	
Nutritional and Metabolic	Liver disease	
	Vitamin D deficiency (with rickets)	Nutritional or sunshine deprivation; gastrointestinal disease
	Calcium deficiency	Maize-based diets
	Copper deficiency	Artificial diets
	Vitamin C deficiency (scurvy)	Nutritional deprivation
Immunologic and Inflammatory	Systemic mastocytosis	
	Rheumatoid arthritis	
	Hyper IgE syndrome	
Neoplastic	Leukemia	Direct invasion of bone
	Neuroblastoma	Direct invasion of bone
Immobilization	Chronic neurogenic	Cerebral palsy and neural tube defects
	Acute neurogenic	Traumatic paraplegia
	Burns	? Cytokine effect
Miscellaneous and Unknown	Thalassemia major	Transfusional hemosiderosis
	Idiopathic juvenile osteoporosis	

ATPase, adenosine triphosphatase

Sillence's classification of OI (Table 2, page 20) is widely used but is being superseded as advances are made in molecular genetics.[5]

The severity of OI depends on the type, and is variable even between individuals in the same family who have the identical genetic defect. Regardless of type, all patients are osteopenic,[6] and their bones are liable to fracture with minimal trauma. In severe OI, fractures may occur in utero. In milder cases, the first fracture may occur at any age up to old age (**10% of infants with mild OI are born with fractures**). Fracture or separation of the epiphysis is rare in OI, and OI is important in the differential diagnosis from trauma due to child abuse. Spinal osteoporosis may begin in the first decade, as can vertebral collapse. Dentinogenesis imperfecta causes the teeth to be grayish blue or brown, with reduced resistance to wear. Abnormalities also occur in other collagenous tissues such as ligaments and the sclerae, which are often blue. **Despite many attempts, no effective nonsurgical therapy exists for OI. Prenatal diagnosis from the genotype of the fetus is possible if the collagen gene mutations in affected family members are known.** Prenatal diagnosis under such circumstances depends on material taken by chorionic villus biopsy performed as early as 8 weeks gestation.[7]

Homocystinuria

Homocystinuria is a recessively inherited disorder causing mental retardation, a distinctive appearance, and the urinary excretion of excess amounts of the sulfur amino acid homocystine. This rare condition has provoked considerable research interest because of the high incidence of 2 very common disorders associated with it: arterial thromboses, a frequent source of morbidity, and osteoporosis. In homocystinuria, the limbs are thin and

Table 2
Sillence Classification of Osteogenesis Imperfecta[5]

OI Type	Fragility	Sclerae	Dental Involvement	Inheritance	Comments
IA	Present	Blue	Yes	Aut dom	Relatively common
IB	Present	Blue	No	Aut dom	Variable severity
II	Extreme	Blue		? dom (germ cell)	Perinatal
III	Severe	Normal	No	? dom (germ cell)	Skeletal deformity
IVA	Present	Normal	Yes	Aut dom	Uncommon
IVB	Present	Normal	No	Aut dom	Variable severity

Aut dom, autosomal dominant

spindly, with a decreased upper:lower segment ratio. Tall stature is present and persists into adult life. Kyphoscoliosis is common, and severe osteoporosis begins in adolescence. There is downward dislocation of the lens, leading to secondary glaucoma, myopia, and retinal detachment.

HORMONAL OSTEOPOROSIS

Hypogonadism

Both androgens and estrogens promote bone anabolism. Osteoblasts bear receptors for both classes of hormones. In both sexes there is a sharp increase in bone mineral content during puberty. **Osteopenia is seen in adolescents and young adults of either sex in a variety of settings of gonadal hormone deficiency.**

Symptomatic osteoporosis used to be common in young women with *Turner syndrome* before estrogen replacement became routine. **Recent data clearly show that with adequate estrogen replacement, osteoporosis can be avoided in Turner syndrome.** It is less clear whether growth hormone, used investigationally to promote growth in these short girls, leads to an increase in bone density before estrogens are administered.

Reduced gonadal hormone output is a hallmark of *exercise-anorexia nervosa amenorrhea* and is also present in many highly trained athletes. There are areas of physiologic overlap between anorexia and athleticism in the anorectic's urge to exercise and the athlete's concern for a trim, muscular, and efficient body. **Both in anorexia and in athletic training, males and females may suffer from diminished gonadal function.** However, the diagnosis is made far more commonly in young women, and more is known about the skeletal consequences in females than males with this syndrome. **Significant osteopenia is common in anorexia**,[8,9] leading to concern that clinically significant osteoporosis might develop in middle age. **However, osteoporotic fractures of the vertebrae or limbs are uncommon in young anorectic girls.** It is hard to tell how much of the osteopenia of anorexia is due to hypoestrogenemia and how much to nutritional deficiency, but analogy with more clearly defined varieties of hypogonadism indicates that hormonal deficiency plays a major role.

By imposing varying loads on the skeleton, exercise promotes bone anabolism. Nevertheless, female athletes and other trained young women, such as ballerinas, lose bone and may become osteoporotic even if they are reasonably well nourished. **This bone loss stems from the negative effects of acquired hypogonadism, which override the benefit from exercise.** In contrast to the situation in anorexia, fractures—particularly cortical microfractures of the lower limbs—are not uncommon. This is related to the great strain imposed upon the limbs by the activities of these girls. Bone loss may not be fully reversed with estrogen treatment.[10]

Young men with *Kallmann's syndrome* and other causes of hypogonadism are often osteopenic. Their reduced bone mass is reversible upon administration of androgens. Despite the presence of androgen receptors on bone cells, doubt has been cast on the role of androgens by the fascinating reports of osteopenia in males who cannot produce estrogens (aromatase deficiency) or who are resistant to estrogens.[11] In both of these unusual disorders, men developed significant osteopenia despite normal testosterone levels.

Glucocorticoid Excess

Cushing's syndrome causes pathologic bone loss in children and inhibits linear growth. The

bone loss can be severe, with frequent limb and vertebral fractures. The cellular causes of corticosteroid-induced osteoporosis relate more to the catabolic effects of glucocorticoids on matrix protein than to any effect on intestinal or renal calcium handling. Although there is no reliable treatment for corticosteroid-induced osteoporosis, attempts at prevention have been made by altering the schedule of corticosteroid administration, eg, alternate-day doses, and by using analogues such as deflazacort, designed to have an improved therapeutic ratio. **Established disease has been treated with vitamin D, calcium supplements, antiresorptive agents, and anabolic agents such as androgens and growth hormone, all to little avail.**

Thyrotoxicosis and Hyperparathyroidism

Endogenous and factitious thyrotoxicosis in childhood may cause osteoporosis if untreated for a long period. Hyperparathyroidism may be associated with bone loss, but the sites of skeletal damage are quite specific and different from those seen in true osteoporosis.

NUTRITIONAL AND METABOLIC DISORDERS

Liver Disease

The liver is involved in calcium metabolism as the site of the first (25-hydroxylation) step in the activation of vitamin D. Additionally, bile production is essential for the normal absorption of both vitamin D and calcium. **As a result, severe bone disease resulting in a combination of rickets (a form of osteomalacia) and osteoporosis can occur in young children with biliary obstruction.**[12]

Vitamin D Deficiency (With Rickets) and Calcium Deficiency

These conditions, the first being common and the second being very rare in industrialized countries, produce specific radiologic changes, mainly affecting the epiphyses. The bending of bone due to bony softening (osteomalacia) is quite different from the pathophysiology of pure osteopenia.

Immunologic and Inflammatory Disease

Cytokines, such as those that signal between cells of the immune system, can influence the relative activities of bone-forming and bone-resorbing cells. Conditions in which cytokine production is deranged, such as systemic mastocytosis, rheumatoid arthritis, and the hyper IgE syndrome, are associated with osteoporosis. **In juvenile rheumatoid arthritis, disuse osteoporosis is combined with corticosteroid effects and a catabolic effect of immune cytokines results, leading to a particularly refractory osteoporosis.**

Neoplastic Diseases

Crush fractures of the vertebrae and pathologic fractures through malignant deposits in appendicular bone occur in leukemia, neuroblastoma, and other malignancies. The radiologic appearances may be patchy, with some areas of the skeleton appearing normal.[13] In other instances, the osteopenia is diffuse. **Malignant osteoporosis of childhood is often painful, providing a strong clue as to the underlying diagnosis.** Excessive bone resorption may lead to hypercalciuria or even hypercalcemia, but the absence of biochemical abnormalities does not exclude malignancy as a cause for childhood osteoporosis.

Immobilization Osteoporosis and Osteoporosis of Miscellaneous and Unknown Causes

Disuse osteoporosis occurs in congenital paraplegia (spina bifida), cerebral palsy, and acquired paraplegia. Mechanical stresses stimulate the formation of new bone and the gain of skeletal mass. Conversely, weightlessness, bed rest, and paralysis lead to bone loss. Lower limb fractures are common in nonambulatory children with spina bifida and cerebral palsy. **Often nutritional factors contribute to the development of bone disease in these multiply handicapped individuals.** A variety of orthopedic approaches may be necessary to deal with the fractures, but correction of the underlying cause is generally not an option.

Acute paraplegia causes rapid bone loss in otherwise healthy growing children, as children have higher bone turnover rates than adults. Osteoporosis of the paralyzed limbs is the rule in such cases, and a major clinical problem is the rapid rise in serum and urinary calcium after injury.

Bone loss after burn injury has been attributed to immobilization. However, **more recent work**[14] **has suggested that biochemical markers of bone formation can remain depressed for years after burn injury.** The cause of such long and lasting

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depression of skeletal function following burn injuries remains to be discovered.

Idiopathic juvenile osteoporosis (IJO) is a term used to describe a severe and rapidly progressive form of osteoporosis seen in the years before puberty.[15] The disease affects both sexes and its cause is unknown. At the onset of puberty, the disease remits and, while residual deformity persists, new growth takes place in the absence of further fractures. IJO is not known to be a familial condition, but the possibility that it is due to an underlying disorder of collagen formation, much like that in OI, cannot be ruled out. **The remission of osteoporosis with puberty is a feature shared by many other types of osteoporosis in adolescents, even those whose causes have nothing to do with gonadal hormone status, such as in corticosteroid-induced osteoporosis and OI.** The natural history of these conditions bears witness to the powerful effect of gonadal hormones on bone.

PEDIATRIC ASPECTS OF ADULT OSTEOPOROSIS

If bone mass increases steadily throughout childhood and the early 20s and is then subject to an inevitable decline, it follows that measures to promote skeletal accretion in youth may limit the effects of bone loss later in life. **The factors contributing to the gain in bone mass in childhood include calcium nutrition,[16] the timing of puberty, other hormonal influences, and innate genetic traits.** A start in unraveling these genetic factors may have been made by the observation that polymorphisms in the noncoding region of the vitamin D receptor gene are predictors of adult bone mass.[17] Doubtless other genetic influences will be discovered since there is certainly a familial component to postmenopausal osteoporosis.[18]

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Abstracts From the Literature

Identification of a Stimulator of Steroid Hormone Synthesis Isolated From Testis

A follicle-stimulating hormone (FSH)-dependent product of the rat Sertoli cell that stimulates Leydig cell function through paracrine mechanisms was identified. A 70-kd protein complex was resolved into 2 proteins of 28 kd and 38 kd. The 28-kd fraction expressed Leydig cell-stimulating activity. The 38-kd protein permitted maximal expression of this activity. The 28-kd fraction also stimulated steroidogenesis in isolated rat granulosa cells and mouse adrenocortical cells. Further studies revealed that the 28-kd fraction was identical to the tissue inhibitor of metalloproteinase-1 (TIMP-1) and the 38-kd fraction to the proenzyme form of cathepsin L/Sertoli cell cyclic protein-2 (CP-2).

TIMP-1 is present in many tissues. Among other functions, it binds to matrix metalloproteinases or interstitial collagenases and influences cell migration, angiogenesis, embryo implantation, and cell growth. The mechanisms by which TIMP-1 stimulates steroidogenesis are as yet unknown. Procathepsin L enters lysosomes through the mannose-6-phosphate receptor (the type II insulin-like growth factor receptor) and is metabolized to cathepsin L, a cysteine proteinase that is involved in prohormone activation, bone resorption, and sperm maturation. Since TIMP-1 contains 6 disulfide bonds, cathepsin L may be involved in full expression of the steroidogenic activity of TIMP-1 by modifying its 3-dimensional structure.

Boujrad N, et al. *Science* 1995;268:1609-1612.

***Editor's comment**: The importance of TIMP-1/cathepsin L in steroidogenesis in humans is uncertain, although human Sertoli cells have been reported to secrete an FSH-responsive factor that stimulates Leydig cell function.[1] Whether this factor may be involved in the physiology of normal adrenarche or in the pathogenesis of such disorders as polycystic ovary syndrome or male limited gonadotropin-independent sexual precocity in some patients remains an issue for future study.*

Allen W. Root, MD

1. Papadopoulous V. *J Clin Endocrinol Metab* 1991;72:1332-1339.

Growth in Full-Term Small-for-Gestational-Age Infants: From Birth to Final Height

This study took advantage of the features of the population in the health-care and school systems of Sweden, where there is low migratory activity, 98% of the children are in school at 17 to 19 years of age, and accurate data on auxologic measures for almost all children from birth through 18 years of age are available. Followed in the study were 3,650 children without dysmorphology; none had any known reason for short stature of pathologic origin.

The aims of the study were: (1) to describe the postnatal growth pattern for small-for-gestational age (SGA) children defined as SGA by either birth weight or birth length <-2 SDS, instead of by birth weight only, as is the usual criterion for SGA; (2) to determine the relative risk of ultimate short stature in children classified as SGA by either definition; and (3) to identify predictors for ultimate short stature in SGA infants by correlating the growth patterns with independent variables such as size at birth, midparental height, length of gestation, and sex.

The entire group was subdivided into 4 groups (Table 1). Group 1, the normal group for birth weight and length (N), consisted of 94.6% of the newborn population. Group 2 (SGA for weight only, SGA_W) consisted of 1.6%. Group 3, which was SGA for both weight and length (SGA_{WL}), consisted of 1.5%. Group 4, which was SGA only for length (SGA_L), was a significant percentage at 2.4%. Rapid growth occurred in the first 6 to 12 months in all groups, but most rapidly in groups 3 and 4. At 2 years of age, 9.9% and 13.4% of groups 2 and 4, respectively, remained <-2 SDS for length. By 18 years of age, there still were 6.4% of group 2 and 7.9% of group 4 who were <-2 SDS for length. Calculations were not given for group 3. The final height SDS for group 2 (-0.4) was the most severely stunted of the groups. The authors reported that at final height, 22% of the short individuals in a clinic for short stature were found to be SGA as defined by birth length; 14% were found to be SGA as defined by birth weight. Birth length SDS and length of gestation were positive predictors of catch-up growth by 6 months of age; only midparental height and birth length were predictors of final height gain by 18 years of age.

Table 1
Characteristics of Birth Size Groups

Classification	N Group 1	SGA_W Group 2	SGA_{WL} Group 3	SGA_L Group 4
Birth length	>-2 SDS	>-2 SDS	<-2 SDS	<-2 SDS
Birth weight	>-2 SDS	<-2 SDS	<-2 SDS	>-2 SDS
Total percentage	94.6%	1.6%	1.5%	2.4%
Percentage <-2 SDS at 2 years		9.9%		13.4%
Percentage <-2 SDS at 18 years		6.4%		7.9%
Δ SDS in first 12 months		0.6 SDS_L	1.2 SDS_L	1.3 SDS_L
Mean final height		-0.4 SDS	-0.4 SDS	-0.8 SDS

SDS, standard deviation scores
SGA, small-for-gestational age
N, normal
SGA_L, SGA defined by birth length
SGA_W, SGA defined by birth weight
SGA_{WL}, SGA defined by birth weight and length

The authors concluded that the majority (>86%) of healthy full-term singleton SGA infants will exhibit catch up in height during the first 6 to 12 months of life and that this is almost independent of whether birth weight or length is used to define SGA. They add that of the SGA infants remaining <-2 SDS at 12 months, about half will be short in final height, thus constituting a high-risk population for persistent short stature.

An intriguing aspect of this study was a determination of the relative risk (RR) for short stature in SGA infants defined by birth length (groups 3 and 4), which was slightly higher (RR = 7.1) than that for SGA infants (RR=5.2) defined by birth weight (groups 2 and 3). However, there is no statistical difference between 7.1 and 5.2. The authors emphasize that SGA infants are usually defined in terms of birth weight alone, and if that definition had been used in this study, group 4 would have been eliminated. Poignantly, omission of group 4 would have had a major influence on the results, especially when the outcome is the postnatal gain in height, as the authors found that the majority (61.7%) of the short newborn population belonged to group 4. They also add that it is difficult to compare the results of this study with those of other studies, as there are differences in definitions of the study populations.

Karlberg J, Albertsson-Wikland K. *Pediatr Res* 1995;38:733-739.

***Editor's comment**: Much has been written about growth and growth prognosis in SGA infants, but never based on data as solid as that presented in this excellent study. The authors have enjoyed the luxury of performing a longitudinal retrospective analysis of data due to the homogeneity of the study population and the supportive structure of very well-organized health-care and school systems. They showed that birth length was a predictor of final height.*

The traditional definition of adequacy of size for gestational age based only upon birth weight is very elegantly challenged here, where birth length is also included. As a result of this variable, a new group of infants is considered within the SGA group, and this was the most prevalent. These are the infants who are short despite having a normal birth weight, labeled here as group 4. The final height SDS of these infants is closest to that of group 3 individuals, traditionally known as symmetric SGA. The latter are usually considered

as having a poorer growth prognosis. The catch-up growth is impressive in short-for-gestational-age babies (groups 3 and 4) during the first 6 months of life, but their final height remains affected. In contrast, babies in group 2 (normal length but low weight) did not show a dramatic catch-up growth but did end up with a better height.

These facts should stimulate us to improve the accuracy of birth length measurements in delivery rooms and nurseries. In many instances, the current practice for length measurements involves very inaccurate techniques.

Fima Lifshitz, MD

Male Pseudohermaphroditism Due to a Homozygous Mutation of the LH Receptor Gene

The investigators report that in 2 siblings with male pseudohermaphroditism associated with Leydig cell hypoplasia (testicular histology characterized by a paucity of interstitial cells and seminiferous tubules primarily composed of Sertoli cells) there was a homozygous G→C transversion at nucleotide 1787 in exon 11 of the luteinizing hormone receptor (LHR) gene, resulting in an Ala593Pro substitution in the sixth transmembrane domain of the LHR. This mutated receptor had normal binding affinity for LH but was unable to transduce an intracellular signal (adenylyl cyclase - cyclic adenosine monophosphate), thus rendering it nonfunctional. This resulted in decreased testosterone production and failure of development of normal male external genitalia. The data demonstrate the embryologic importance of the LHR for normal Leydig cell differentiation, proliferation, and function.

Kremer H, et al. *Nature Genet* 1995;9:160-164.

***Editor's comment**: The mutation Ala593Pro in the LHR renders it nonfunctional. This is of interest because of its proximity to many other mutations of the LHR that render it constitutively active. These are presented in Table 1.*

Table 1
Mutations in Familial Male-Limited Isosexual Precocity

cDNA	Nucleotide Change	Amino Acid Change	Location
1624	A→C	Ile542Leu	TM domain V
1691	A→G	Asp564Gly	Third intracellular loop
1713	G→A	Met571Ile	TM domain VI
1715	C→T	Ala572Val	
1723	A→C	Ile575Leu	
1725	G→A	Met575Ile	
1730	C→T	Thr577Ile	
1732	G→T	Asp578Tyr	
1733	A→G	Asp578Gly	
1741	T→C	Cys581Arg	
1745	A→G	Asp582Gly	

TM = transmembrane

The mutation at amino acid 593 is distal to the mutations leading to constitutive activation of the LHR. This suggests that between amino acids 583 and 592 a transition point is located that alters the relationship between the LHR and its associated G_s protein. Of interest is whether this mutated LHR is able to activate other signal pathways, such as phospholipase C through G_q protein.

Allen W. Root, MD

***2nd Editor's comment**: The clinical aspects of this type of male pseudohermaphroditism, as reviewed in this article, also are worthy of comment.*

The probands were the 46,XY products of first cousins. They had sexual infantilism; female external genitalia; short blind vaginas; no uterus or fallopian tubes; low testosterone levels, which failed to rise with human chorionic gonodotropin (hCG) stimulation; markedly elevated LH but normal follicle-stimulating hormone levels; and testes with Sertoli cells but no mature Leydig cells. Wolffian duct tissue (epididymis and vas deferens) was found, which was surprising since it is found only in the presence of Leydig cell androgens at some point in fetal development. This supports the concept that androgen synthesis in Leydig cells is initiated early in fetal life, independent of LH or hCG synthesis. The authors also conclude that the data and findings further demonstrate that at a later fetal stage the absence of a functional LHR interferes with Leydig cell proliferation and maturation. No abnormal female sex characteristics have been noted in sisters of patients with this form of pseudohermaphroditism. This is consistent with experimental results that indicate absence of a functional ovarian LHR until after birth. Several sisters of reported patients have had amenorrhea, which reflects the need for LHR to be present for normal menstruation.

Nine references are given concerning patients with absence of Leydig cells or insufficient Leydig cell differentiation occurring as an autosomal recessive condition. The phenotypes range from an extreme form of male hermaphroditism to milder forms in which males present with hypergonadotropic hypogonadism and a micropenis. Testicular LH binding was decreased or absent in some studies; this could be either the cause or consequence of Leydig cell hypoplasia. This article is well worth reviewing in its entirety for those interested in the multiplicity of genetic and/or auxologic and related biochemical alterations in male pseudohermaphrodites.

Robert M. Blizzard, MD

Short-Term Effect of Testosterone Treatment on Reduced Bone Density in Boys With Constitutional Delay of Puberty

This study demonstrates: (1) that boys with constitutional delay in growth and sexual maturation (CDGP) have decreased bone mineralization beyond that which can be explained by their short stature or delayed bone age, and (2) that in subjects who have received testosterone for 6 months there is increased bone mineralization 6 months later compared with untreated subjects. Bone mineral density (BMD; grams per square centimeter) and bone mineral content (BMC; grams per centimeter) were determined in the nondominant radius by single photon absorptiometry (SPA) in 17 white males with CDGP. BMD and BMC were decreased relative to control data for chronologic age (14.6 ± 1.0 years), height age (11.6 ± 1.6 years), and bone age (11.9 ± 1.6 years). Eight boys received testosterone depot, 100 mg/mo intramuscularly, for 6 months. Six months later (12 months after initiation of testosterone therapy), height, weight, and sexual maturation of the testosterone-treated youths were greater than that of the 9 control subjects, and BMD and BMC had increased substantially as well ($P<0.001$). BMD increased +26.2% ± 13.6% in testosterone-treated boys versus +0.54% ± 8.7% in nontreated subjects. BMC increased +41.1% ± 28.8% in testosterone-treated boys and +5.1% in the untreated subjects. The investigators concluded that boys with CDGP have decreased bone mineralization that is disproportionately greater than that related to their short stature and delayed skeletal maturation, and that short-term administration of testosterone increases bone mineralization.

Bertelloni S, et al. *J Bone Miner Res* 1995;10:1488-1495.

***Editor's comment**: Approximately 30% of adult lumbar spine BMC is accumulated within a 3-year peripubertal interval, and half of the increase in BMC that occurs during adolescence reflects growth in bone size rather than increase in bone density. Young adult males with CDGP have lower spinal BMD than do control subjects with earlier adolescence, emphasizing the importance of the timing of adolescence as well as the secretion of sex hormones themselves for this process. Whether there are adverse clinical consequences of the lower BMD of CDGP subjects is not known. In the present report, compact bone mineralization has been measured only at the radius by SPA. It will be important to confirm these findings by more extensive evaluation of skeletal mineralization, including sites of trabecular bone such as the lumbar spine and hip, employing dual-energy X-ray absorptiometry. Whether decreased bone mineralization in the male with CDGP should lead to more aggressive therapy is uncertain as yet.*

Allen W. Root, MD

Computer-Aided Skeletal Age Scores in Healthy Children, Girls With Turner Syndrome, and in Children With Constitutionally Tall Stature

The aims of the present study were: (1) to evaluate the reliability of a computer-assisted bone age scoring system in healthy children; (2) to compare this method against a manual rating system in healthy children, as well as in subjects with 2 specific entities, manifested by short and tall stature, respectively; and (3) to determine whether a shortened version of the bone age scoring system might substitute for the original long version. Reference curves for bone maturation in Turner syndrome (TS) and constitutional tall stature (CTS) determined by the computerized system are presented.

Three groups of individuals—healthy children, girls with TS, and children with CTS—underwent bone maturation evaluation. Evaluations were conducted manually using the 13b model of the Tanner-Whitehouse method (TW-RUS), and by the long (13b) and the shortened (6b) models of the computer-aided skeletal age scoring system (CASAS), a transformation of the TW-RUS method into a computerized image analysis system.

As evaluated by the percentage of equal ratings on duplicate (within-observer as well as between-observer) assessments, reliability was high (±90%) in healthy children and similar to those obtained by the manual ratings. The comparability of CASAS (both 13b and 6b models) with the manual rating system was assessed by calculating the correlation coefficients and evaluating the average and the limits of the range of agreement by a method described by Bland and Altman. Although some of the mean differences of methods were statistically significant, they were not clinically significant, as they were <0.4 bone age year. Up to 8% of manual insertions occurred in all groups. The percentage was lower in the 6b model than the 13b model, particularly in the CTS children. This suggests that the 6b model of CASAS may have a comparable level of reliability, while introducing a smaller degree of inconsistency.

The authors conclude that CASAS is applicable in TS and CTS. Their data support the use of the 6b model of CASAS, as it is less time-consuming and labor-intensive and provides data almost as reliable as the manual ratings.

Van Teunenbroek A, et al. *Pediatr Res* 1996;39:360-367.

***Editor's comment:** This challenging article compares CASAS scores, particularly those obtained with the 6b model, with manually obtained TW-RUS scores, in the evaluation of the bone maturation in 3 populations of children. Although some*

inconsistencies were noted, the methods were comparable. The main advantage of the computerized method over the manual rating method is that it uses a continuous scale instead of an interval scale. This diminishes the error of the interpretation of maturity stages, as the difference of 1 interval stage in the rating of a particular bone may result in an increase of 0.3 bone age years. The 6b model was less time-consuming than the 13b model, and yielded acceptable readings of bone maturation. The main disadvantage of CASAS is the need for special equipment (hardware and software), which increases the cost. The curves for bone maturation in TS girls and CTS children presented in this article will enhance our understanding of the dynamics of growth, whether spontaneous or in response to specific therapeutic modalities. The procedure has been technically described by Tanner and Gibbons (J Pediatr Endocrinol *1994;7:141-145), in an article that is highly recommended to those of you who wish to review the details of the system and its principles.*

Fima Lifshitz, MD

Intranasal Administration of GHRP Hexarelin Accelerates Growth in Short Children

The investigators administered the growth hormone-releasing peptide (GHRP) hexarelin (His-D-2-methyl-Trp-ALa-Trp-D-Phe-Lys-NH_2) to 8 short prepubertal children (7 males, 1 female; 5.3 to 11.6 years of age). All subjects had normal growth hormone (GH) secretion (>10 ng/mL) in response to provocative stimulation as well as a substantial increase in GH concentrations (>20 ng/mL) following 1 intranasal inhalation of hexarelin (20 µg/kg). Hexarelin (60 µg/kg/dose) was administered intranasally 3 times daily while the children were recumbent. Mean growth rate increased from 5.3 ± 0.8 cm/y to 8.3 ± 1.7 cm/y during the first 6 to 8 months of therapy (P<0.001). Skin-fold thickness declined and head circumference increased during therapy. Serum levels of insulin-like growth factor 1 (IGF-1), inorganic phosphate, and alkaline phosphatase increased during administration of hexarelin. No adverse local or systemic clinical or biochemical events were recorded during this treatment period. The authors concluded that, over the short term, intranasal hexarelin accelerates growth in short children with intact GH secretion.

Editor's comment: *The natural compound whose biologic activity is mimicked by the various synthetic GHRPs is unknown. GHRP acts through a somatotrope receptor that is separate from that for GH-releasing hormone (GHRH) and through a different intracellular signaling pathway (GHRH-adenylyl cyclase/cyclic adenosine monophosphate; GHRP-phosphoinositol). The primary site of action of the GHRPs may be within the hypothalamus rather than directly at the pituitary, as they are inactive in the absence of GHRH. GHRPs are active when administered intravenously, subcutaneously, intranasally, and orally. The present report demonstrates the short-term effects of GHRP administered intranasally. We may anticipate that these agents will also be active during short- and long-term oral administration. If experience demonstrates the safety and effectiveness of oral GHRP, yet another therapeutic agent may be available for the management of the carefully selected short, GH-sufficient child.*

Allen W. Root, MD

Laron Z, et al. *Clin Endocrinol* 1995;43:631-635.

Nondisjunction in Human Sperm: Evidence for an Effect of Increasing Paternal Age

It is well established that increased maternal age is associated with an increased risk of chromosomal trisomy in offspring, ie, the maternal age effect. In contrast, the existence of a paternal age effect has been controversial, with most epidemiologic evidence favoring the absence of such an effect. One of the difficulties has been separating paternal from maternal age effect.

Griffin et al have taken a different approach to the question of paternal age effect. They directly analyzed sperm. Sperm are normally monosomic; they contain only 1 copy of each autosome plus an X or a Y chromosome, ie, 23 X or Y. The authors used fluorescent in situ hybridization (FISH) to count the number of X, Y, and number 18 chromosomes in about 400,000 individual sperm from 24 men, ranging in age from 18 to 60 years. By using probes for both the X and Y chromosomes and chromosome 18, they could distinguish between disomy involving 1 chromosome and diploidy involving a whole complement of chromosomes. Moreover, in cases of disomy for the sex chromosomes, they could distinguish between nondisjunction that occurred during the first meiotic division, which would produce disomic sperm carrying both an X and a Y chromosome, and nondisjunction that occurred during the second meiotic division, which would produce disomic sperm carrying 2 X or 2 Y chromosomes. When such sperm fertilize normal ova, trisomic embryos would be produced containing 47,XXX, 47,XXY or 47,XYY chromosome complements.

The results showed that there was an approximate doubling of nondisjunction for the 3 chromosomes considered together when sperm from men 18 to 29 years were compared with sperm from men 50 to 60 years of age. The numbers were small: 0.11% for the former and 0.27% for the latter. Most of the disomy involved the X and Y chromosomes, with disomic sperm containing XY outnumbering disomic sperm containing XX or YY by about 2:1.

The authors concluded that nondisjunction does occur during male meiosis. It mainly involves sex chromosomes and increases with age, approximately doubling between the ages of 20 to 60 years. However, they point out that the risk for producing trisomic offspring is low: In men over the age of 50 years, only 0.27% of sperm were disomic for the X and/or Y chromosome. The authors also caution that it is not known if disomic sperm compete equally with normal (monosomic) sperm for fertilization. If not, then the clinical relevance of disomic sperm may be moot.

Griffin DK, et al. *Hum Mol Genet* 1995;4:2227-2232.

***Editor's comment**: This is the first direct evidence that meiotic nondisjunction increases with age in males. As the authors mention, the effect is small, which probably explains why epidemiologic studies have failed to detect a paternal age effect. They rightfully point out that their results provide little basis for suggesting that older men, like older women, be offered prenatal testing for age-related trisomy. In reality, however, since older women are usually married to older men, such testing may be undertaken anyway.*

William A. Horton, MD

A Double-Blind, Placebo Controlled Study of the Effects of Low-Dose Testosterone Undecanoate on the Growth of Small for Age, Prepubertal Boys

Twenty-three short prepubertal boys (11 to 14 years of age) with heights at or below the 3rd percentile were randomized into a double-blind study comparing the effects of oral testosterone undecanoate (TU; 20 mg qd for 6 months) versus placebo on various growth parameters. Treatment was preceded and postluded for 6-month periods. The aim was to assess whether very low doses of TU could accelerate growth velocity (GV) without unduly advancing bone age (BA) in boys with constitutional delay of growth and puberty (CDGP).

The investigators reported that 11 boys taking TU showed a significantly greater GV compared with 12 boys receiving placebo (GV = 5.84 vs 3.38 cm/y), a difference of 2.46 cm/y attributed to 6 months of TU treatment. The effect on BA, axillary and pubic hair, lean body mass, and testicular volume was negligible. Nocturnal growth hormone (GH) concentrations measured over an 8-hour period (every 20 minutes) did not change with treatment. Measurement of serum testosterone, before and following testosterone administration in the morning, revealed an average 10-fold increase at 1-hour postingestion. Within 8 hours, the increase fell to less than 4-fold. There was a projected fall to base level by 16 hours. The authors appropriately emphasized that the efficacy of anabolic or sex steroids in promoting short-term growth and increasing final height is as yet unproven, and that carefully designed, controlled, prospective trials to determine the optimal regimen for growth acceleration would be of great potential therapeutic benefit to many children.

Brown DC, et al. *Arch Dis Child* 1995;73:131-135.

***Editor's comment**: The authors are to be commended for designing a well-planned study of the type needed for the stated purposes. Unfortunately, this study was too brief to provide adequate information regarding how best to treat CDGP patients with TU. Specifically, 6 months of treatment that provided small alterations in GV is prone to quantitative misinterpretation. A GV increase of approximately 1.25 cm in 6 months (projected to be at a rate of 2.5 cm in 12 months) is at great risk of being in error. A 0.5 cm error at one measurement and a 0.5 cm error in the opposite direction 6 months later will produce an error of 1.0 cm/y or 2.0 cm/y projected. Some smoothing of the error may occur when groups of children are studied, but the error remains significant. This is not to say that low doses of TU do not increase GV. Nevertheless, errors in measurements taken over only a 6-month period of treatment can lead to erroneous conclusions. Even if one accepts that there may be no significant error, 6 months of treatment yielding a gain of 1.25 cm, is relatively insignificant in producing the alterations that are therapeutically effective in boys with CDGP. Therefore, a 12 month study would have been much more useful.*

Another possible error in the protocol was the morning administration of TU and the nocturnal measurement of GH concentrations 12 to 16 hours later, when serum testosterone levels have fallen to essentially the projected level of untreated boys. TU given in the morning may have been associated with increased GH levels during the day that went undetected because GH was not measured at that time.

Another advantage of a 12 month study is that BA acceleration often requires more than 6 months of observation to be recognized. Twelve months of treatment might have demonstrated inappropriate advancement of BA. The authors are invited to write a letter of rebuttal if they wish.

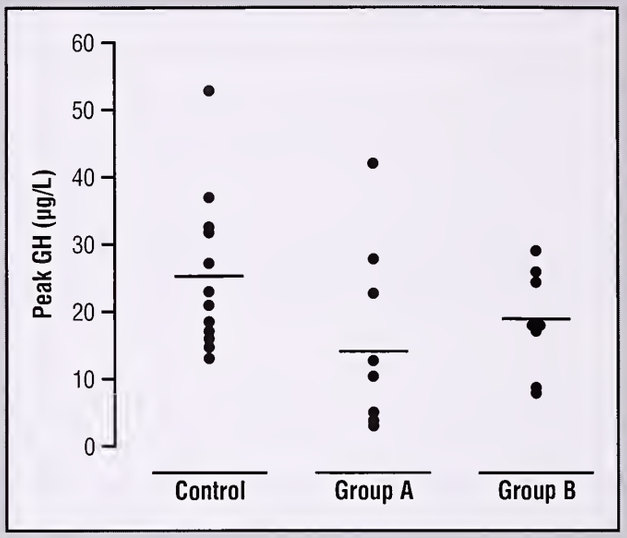
Robert M. Blizzard, MD

Plasma Growth Hormone Response to Growth Hormone-Releasing Hexapeptide (GHRP-6) in Children With Short Stature

Pombo et al measured growth hormone (GH) levels every 15 minutes (for 90 minutes) following an intravenous bolus of the synthetic hexapeptide GHRP-6 (1 µg/kg). This agent is one of a group of synthetic compounds that have been shown to release GH by a non–GH-releasing hormone (GHRH)-dependent mechanism. The authors tested whether GHRP-6 could be used to diagnose GH deficiency in children with short stature. Three groups of children were studied. The first group (A) included 10 children with idiopathic GH deficiency, as determined by failure of GH levels to rise to 10 µg/L following provocative stimulation. The second group (B) included 8 children with normal GH response to provocative stimuli but with markedly reduced 24-hour integrated GH concentrations. The third group (C) included 12 normal prepubertal children (Figure 1).

All 10 patients in group A showed variable responses to GHRP-6; 50% showed a response > 10 µg/L. In group B, 6 of 8 subjects showed a GHRP-6 response > 10 µg/L. Although there were differences between mean GH secretion in response to GHRP-6 in group A patients compared with normal children, the results suggest that GHRP-6 stimulation is not an adequate method for diagnosing idiopathic GH deficiency.

Figure 1
Peak GH Responses to GHRP-6 of Each Patient by Diagnostic Grouping



Pombo M, et al. *Acta Paediatr* 1995;84:904-908.

Editor's comment: *Interpretation of these data is that approximately 50% of children diagnosed as having idiopathic GH deficiency by provocative stimuli are able to release GH in response to GHRP-6. Furthermore, 75% of the children with neurosecretory GH deficiency release GH in response to GHRP-6. The authors concluded that GHRP-6 is of no value in diagnosing idiopathic GH deficiency. Perhaps a more provocative conclusion is that the data demonstrate that many children diagnosed with idiopathic GH deficiency have defects in GH secretion rather than GH synthesis. The mechanism of action of GHRP-6 remains controversial. Both a direct effect at the pituitary and/or the hypothalamus by way of somatostatin or GHRH have been suggested. Studies with GHRP-6 have the potential to reveal important information concerning the normal mechanism of GH synthesis and secretion.*

William L. Clarke, MD

Does Linear Growth Occur Continuously or as "Saltatory" Growth?

Lampl et al[1] reported that in daily, semiweekly, or weekly measurements of crown-heel length in 31 normal infants followed for 4 to 15 months, linear growth proceeded in a start-stop manner; that is, the infant grew at rapid rates for a brief period of several days, followed by prolonged intervals (average 12 days, but as long as 63 days) without any increase in length. Thus, growth in infancy was not continuous but composed of intervals of stasis and rapid growth, a pattern termed saltatory growth. Heinrichs et al[2] challenged this observation. They measured crown-heel (Harpenden-Holtain infantometer), knee-heel (from photographs), head circumference, and weight of 5 infants (1.6 to 4.2 months) at the same hour of every day for 1 month. These investigators concluded that their data indicated the infants grew continuously in all aspects. By their analyses of direct inspection of individual growth curves, frequency distribution of growth velocities, cumulative probability plots, and correlation of crown-heel and knee-heel growth rates, they could find no evidence for saltatory growth and concluded that growth in infancy was continuous. Lampl et al[3] rebut the observations of Heinrichs et al. They point out the inter- and intra-individual variation in growth pattern in infants in their own data and that 1 month of measurements may have been insufficient to observe saltatory growth in the infants reported by Heinrichs et al. Furthermore, Lampl and colleagues reanalyzed the Heinrichs data and concluded that these infants did indeed display a saltatory growth pattern. For these and other reasons, Lampl et al reject the criticisms of Heinrichs et al.

1. Lampl M, et al. *Science* 1992;258:801-803.
2. Heinrichs C, et al. *Science* 1995;268:442-445.
3. Lampl M, et al. *Science* 1995;268:445-447.

Editor's comment: *The implications of the 2 different models of growth—continuous versus saltatory—for the regulation of mitogenesis and growth are significant. This writer finds it difficult to conceptualize a regulatory system in which cellular growth completely ceases and then resumes. On the other hand, a system that modifies the rates of cellular growth (but not to zero) is less difficult to conceptualize because this is observed clinically in the growth of normal infants, children, and adolescents, as well as during and after intervals of illness or suboptimal nutrition. I would prefer to consider the periods of absent growth observed by Lampl et al[1] as intervals of such slow cellular replication that the measurement instruments utilized to record growth were too insensitive to recognize them—thus merging the concepts of continuous and saltatory growth.*

Allen W. Root, MD

A Novel Transcriptional Activator Originating From an Upstream Promoter in the Human Growth Hormone Gene

Editor's comment: *The finding of a "gene within a gene" is intriguing. By analogy to large proteins that may be precursors for several peptides, eg, proopiomelanocortin, it is likely that other genes will be identified with similar construction. It is of interest to speculate that growth hormone-derived transcriptional activator (GHDTA) may be a transcription-activating factor for proopiomelanocortin; alternatively, it might serve as a repressor of human growth hormone (hGH) gene transcription in corticotropes. Now that you have read this comment, please read the abstract that follows.*

Allen W. Root, MD

Labarrière and coworkers identified a second gene product that begins in the upstream promoter region of the hGH gene and includes all of the first and part of the second exon of the hGH gene. The major transcription-activating factor for the hGH gene is Pit-1; this factor binds to upstream bases -130 to -105 and -92 to -65 to initiate gene transcription for hGH mRNA. Between bases -294 and -177 is a sequence that also has the structure of a transcription-promoting region. These investigators cloned the mRNA transcribed from this secondary promoter region, which begins at base -151 in the hGH gene and ends in the middle of exon 2 of the hGH gene at a stop codon, the result of a frameshift. This mRNA encodes a protein of 107 amino acids (molecular weight = 11.4 kd). Expression of mRNA for hGH is confined to the pituitary somatotrope, whereas the protein product of the secondary mRNA is detectable in pituitary corticotropes and placenta. The second protein has been termed GHDTA because it acts as a transcription-activating factor in cells transfected with reporter genes. GHDTA has homology with a liver-specific transcription factor and contains potential protein kinase C-dependent phosphorylation sites. The investigators suggest that GHDTA might be a DNA-binding transcription factor or an activator of a transcription factor.

Labarrière N, et al. *J Biol Chem* 1995;270:19205-19208.

2nd Editor's comment: *A change in* GGH's *usual format was made for this abstract and the editor's comment precedes the abstract. This was done so that readers may better interpret the abstract.*

Robert M. Blizzard, MD

Insensitivity to Anti-Müllerian Hormone Due to a Mutation in the Human Anti-Müllerian Hormone Receptor

Müllerian-inhibiting substance (MIS), a product of the Sertoli cell, causes regression of müllerian duct development and prevents formation of the fallopian tubes, uterus, and upper third of the vagina in the normal male. Abnormalities in the production of MIS lead to the persistent müllerian duct syndrome (PMDS) of müllerian duct structures in phenotypic and genetic males. The present investigators have isolated and characterized the human MIS receptor and have identified a patient with PMDS due to an abnormality in the gene for this receptor. This patient thus has end-organ insensitivity to MIS. This gene is situated on chromosome 12q13 and is composed of 11 exons that encode a mature protein of 573 amino acids. Exons 1 through 3 encode the signal sequence (17 amino acids) and extracellular domain (127 amino acids); exon 4 encodes the single transmembrane domain (26 amino acids); and exons 5 through 11 encode the intracellular domain (403 amino acids), which has serine/threonine kinase activity. The human MIS receptor is homologous to that of the rat (78.5%) and rabbit (82%). In addition to the testicular Sertoli cell, RNA for MIS and its receptor is expressed in the normal ovary and some granulosa cell tumors.

In a 3-month-old boy with PMDS, the AMH gene was normal, and serum AMH was easily measurable. Analysis of the MIS receptor gene revealed a guanine to adenine (G→A) transition at a guanine-thymine (GT) dinucleotide at the splicing donor site of the 5' end of the second intron. This led to 2 abnormalities of gene transcription: (1) loss of exon 2 (exon skipping); and (2) substitution of aspartate for glycine at amino

acid 78 and the insertion of 4 extra amino acids from the first 12 bases of intron 2 into the 3' end of exon 2 (intron inclusion). Either aberration within the extracellular domain is expected to lead to abnormalities of ligand binding and hence hormone action.

Imbeaud S, et al. *Nature Genet* 1995;11:382-388.

Editor's comment: *MIS is a member of the transforming growth factor-6 (TGF-6) family. The MIS gene (chromosome 19p13.3) contains 5 exons and encodes a protein of 560 amino acids (including a leader sequence of 24 amino acids).*[1] *It is active as a disulfide-linked homodimer. PMDS has been associated with both normal and subnormal or absent production of AMH. In the latter group, abnormalities in the MIS gene have been detected, including deletions, nonsense mutations associated with stop codons, frameshift mutations leading to downstream stop codons, point mutations leading to instability of the protein molecule, and intronic splice donor point mutations.*[1,2] *The majority of abnormalities have been found in exons 1 through 3. Identification of the receptor for MIS and documentation of its abnormality in a patient with PMDS provide further evidence of the importance of these proteins in male sexual differentiation. MIS is also produced by granulosa cells and is involved in the regulation of ovarian function. In the article by Imbeaud et al, PMDS results not from a mutated gene for MIS but a gene for its receptor. Again, the same apparent syndrome may be the same syndrome but of different gene origin.*

Allen W. Root, MD

1. Josso N, et al. *Rec Prog Horm Res* 1993;48:1-59.
2. Imbeaud S, et al. *Hum Mol Genet* 1994;3:125-131.

No Reduction in Birth Weight in Phenylketonuria

A registry of all known children with phenylketonuria (PKU) born in the United Kingdom from 1964 onward allows the definition of growth parameters and natural history. The birth weight, sex, social class, gestational age, disease severity, and birth date were all taken into consideration when determining norms and averages. Data were available for 1,886 infants. The mean birth weight for PKU infants born in the United Kingdom was 3,306.7 g and the median 3,337 g. There are no significant differences from other births in the United Kingdom and PKU individuals show a similar pattern to the normal population.

Tillotson SL, et al. *Eur J Pediatr* 1995;154:847-849.

Editor's comment: *It is nice to have a proper natural history study that facilitates assessment of the natural history of a disorder. This PKU data gave completely normal growth findings in the affected newborn. This work is important since there has been a recent report suggesting impairment was already present at birth.*

Judith G. Hall, MD

A Gene (PEX) With Homologies to Endopeptidases Is Mutated in Patients With X-Linked Hypophosphatemic Rickets

The gene for familial X-linked hypophosphatemic rickets (FHR) has been localized to chromosome Xp22.1. By positional cloning, the present investigators have detected 4 partial deletions and 3 mutations (from a total of 150 families studied) in a gene in this region that is homologous to several endopeptidases such as neutral endopeptidase, endothelin-converting enzyme-1, and the Kell antigen. The deletions ranged in size from <1 to 55 kb; the mutations included loss of a dinucleotide, resulting in a frameshift, and 2 point mutations leading to exon skipping. This gene has been termed *PEX* (phosphate-regulating gene with homologies to endopeptidases on the X chromosome). As do other members of the neutral endopeptidase family, *PEX* has many small exons, a short cytoplasmic amino-terminal domain, a transmembrane segment, and a large extracellular carboxyl-terminal region with a zinc-binding motif and 7 conserved cysteine residues. The investigators hypothesize that the *PEX* endopeptidase is important for processing a circulating factor that regulates function of the sodium-phosphate cotransporter (whose gene is situated on chromosome 5q13). Loss of this endopeptidase would result in an inactive phosphate regulatory factor and decreased renal phosphate resorption.

The HYP Consortium. *Nature Genet* 1995;11:130-136.

Editor's comment: *Identification of a defective gene coding for an endopeptidase as the candidate gene for FHR leads to additional questions. For example, what is the target protein for* PEX *endopeptidase action and where is its gene located? How does this protein affect activity of the sodium-phosphate cotransporter? In addition, it introduces the probability that there are abnormalities in this and other factors that also lead to hyperphosphaturia, hypophosphatemia, and metabolic bone disease. Indeed, autosomal recessive and autosomal dominant forms of hypophosphatemic rickets and hypophosphatemic bone disease have been described that may involve these other proteins.*

Allen W. Root, MD

Ob/Ob and Db/Db Gene(s), Obesity, and Sterility, and Relationships to Leptin

Editorial introductory comment: *Grouping of related abstracts often enhances understanding of an entity or related entities. The story of genetic obesity has complexities that prompt grouping of the following abstracts by Dr. Root. For purposes of orientation, the following introductory remarks to the abstracts are given:*

The ob/ob obese mouse is genetically deficient in the production of the ob gene product, leptin. Leptin administration produces loss of weight and decreases appetite in ob/ob mice. The db/db obese mouse, which has a very similar phenotype to the ob/ob mouse, is not responsive to leptin. Therefore, a receptor defect is apparently present. Different genes have been thought to be involved in the 2 strains. New data referred to below suggest that the same gene may be responsible. We previously learned that identical auxologic phenotypical syndromes such as GH deficiency and Laron syndrome can be of different molecular origins. We are learning now that different components of the same gene can be responsible for the almost identical phenotypic ones, but syndromes that differ in response to a therapeutic agent.

Robert M. Blizzard, MD

Correction of the Sterility Defect in Homozygous Obese Female Mice by Treatment With the Human Recombinant Leptin

The obese female mouse that is homozygous for the ob/ob mutation leading to a decrease in the synthesis of leptin also is sterile. The infertility of these animals is due to dysregulation of hypothalamic-pituitary function that is unrelated to body weight per se, because reduction of body weight to normal values does not restore fertility. There are low levels of reproductive hormones in the ob/ob animal, while the ovaries of these animals are normally responsive to gonadotropins. The present investigators demonstrated that administration of leptin (10 µg/g of initial body weight per day intraperitoneally for 30 to 42 days) resulted in a decline in body weight by 40% to 48%. When mated with normal male mice, all (n=6) female mice copulated and became pregnant (leptin administration was continued at 5 µg/g/d). All (n=28) of the pups that were delivered died by 2 days of age because the mothers did not suckle their young. In another experiment, 6 ob/ob female mice were treated with leptin and lost weight, mated, conceived, and delivered pups immediately, after which the number of pups was purposely decreased to 3 pups/litter in the 2 dams who were able to suckle. These animals survived, as did the offspring that were transferred to normal foster dams. Interpretation of the data are that (1) leptin administration to ob/ob homozygous female mice leads to weight loss, copulation, ovulation, pregnancy, and parturition that is independent of weight loss alone; and (2) lactation is only partially restored by leptin administration, either because of defective breast development in this strain of mice or because leptin adversely affects mammary development/function in the pregnant female ob/ob mouse.

Chehab FF, et al. *Nature Genet* 1996;12:318-320.

Editor's comment: *This paper reports data that support the suggestion that leptin alters hypothalamic-pituitary-ovarian function, permitting ovulation, conception, and parturition in animals with defective synthesis of this fat-derived peptide. (Further studies of the effect of leptin on the secretion of LH, FSH, prolactin, the sex hormones, and breast function must be carried out in order to determine the physiologic mechanisms that underlie the clinical effects of leptin in these animals.) Thus, in addition to its effects on hypothalamic regulation of appetite, leptin may also influence the synthesis/secretion of gonadotropin releasing hormone (GnRH) and/or the pituitary gonadotropins directly. The relationship between body fat content and the reproductive endocrine system, particularly in females, has long been known (eg, the "critical weight" hypothesis of Frisch, which correlates weight loss and amenorrhea accompanying the weight loss of dieting, illness, or excessive exercise), but the mechanism of this regulatory effect has not been identified. Perhaps leptin is the messenger traveling from body fat to the hypothalamus that influences GnRH synthesis/release. Very possibly leptin may play a fundamental role in normal adolescent sexual maturation, as well as in a number of states of energy deprivation.*

Allen W. Root, MD

Evidence That the Diabetes Gene Encodes the Leptin Receptor: Identification of a Mutation in the Leptin Receptor Gene in db/db Mice

The investigators identified an abnormality in the hypothalamic receptor for the fat-derived, anorectic OB peptide (leptin) in the db/db mouse, which is responsible for the phenotype of this animal. The phenotype is similar to that of the ob/ob mouse and characterized by early-onset obesity, insulin resistance, and susceptibility to diabetes. In the normal mouse there are short and long forms of leptin receptor (designated OB-R) that arise by alternative splicing of transcripts. The short OB-R has extracellular leptin-binding transmembrane and intracellular domains of 816, 23, and 34 amino acids, respectively. The long OB-R has an intracellular domain of 302 amino acids. The latter results from the inclusion of an exon that is not transcribed in the OB-R short form. It is the long form of the OB-R that probably is involved in intracellular signaling. It is related to the class I group of cytokine receptors and contains motifs for interaction with janus kinase (JAK) and the signal transducer and activator of transcription (STAT). The db/db mouse pathologically expresses only the short form of the OB-R. It does so because of a mutation (G→T) in the OB-R gene that creates a new splice site that leads to the incorporation of 106 extra nucleotides in the transcript of the OB-R gene, including a stop codon that results in premature termination of the long intracellular portion of the OB-R.

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Chen H, et al. *Cell* 1996;84: 491-495.

Editor's comment: *Recent papers by Lee et al[1] and Chua et al[2] add further information to the genetic defect in the db/db mouse. Lee et al reported identical findings to those of Chen et al in the db/db mouse; they also observed 9 normal splicing variants of OB-R expressed in mouse brain, hypothalamus, adipose tissue, testes, and heart. Chua et al reported that the OB-R gene and those encoding the defects in the db/db mouse and fa/fa (Zucker fatty) rat are the same. Interestingly, the OB-R also is expressed in the ovaries (Chen et al). The human OB-R has been localized to chromosome 1p31.*

Allen W. Root, MD

1. Lee G-H, et al. *Nature* 1996;379:632-635.
2. Chua SC Jr, et al. *Science* 1996;271:994-996.

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Activating Mutations in G Protein-Coupled Signaling Pathways as a Cause of Endocrine Disease

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Unraveling the mechanisms by which extracellular stimuli activate intracellular signaling pathways is an extremely active area of research. Many stimuli, including photons, hormones, neurotransmitters, odorants, proteases, and ions, act through a group of membrane-spanning cell surface receptors that are coupled to guanine nucleotide-binding proteins (G proteins) to modulate the activity of cellular effectors.[1] In the last few years, mutations in the genes encoding components of these signaling pathways have been shown to cause human disease.[2-4] Identification of these naturally occurring mutations not only has value in defining the molecular basis of disease, but also has accelerated progress in understanding the fundamental mechanisms by which G protein-coupled signal transduction occurs. Loss-of-function mutations have been described in several endocrine diseases characterized by hormone resistance, such as pseudohypoparathyroidism type Ia ($G_s\alpha$), hereditary glucocorticoid deficiency (corticotropin receptor), and nephrogenic diabetes insipidus (V2 vasopressin receptor). This review will focus on those endocrine diseases that have been shown to be due to gain of function mutations, ie, where inappropriate signaling occurs in the absence of an agonist (Table 1).

Table 1
Endocrine Diseases Caused by Activating Mutations in G Protein-Coupled Pathways

Gene Mutation	Disease
G protein α subunit	
G_s(*gsp*)	Somatotrope and thyroid adenomas; McCune-Albright syndrome
G_s(Ala366→Ser)	Combined testotoxicosis and pseudohypoparathyroidism type Ia
G_{i2} (*gip*)	Ovarian and adrenocortical tumors
Receptor	
Luteinizing hormone	Testotoxicosis
Thyroid-stimulating hormone	Thyroid adenoma and hyperplasia
Ca^{2+}	Autosomal dominant hypocalcemia
Parathyroid hormone	Jansen's disease (metaphyseal chondrodysplasia)

G PROTEIN AND RECEPTOR SIGNALING

All members of the G protein-coupled receptor (GPCR) family are predicted to share a common "serpentine" structure: a bundle of 7 α-helical hydrophobic regions (TM1 through TM7) connected by alternating extracellular (e1 through e3) and intracellular (i1 through i3) loops (Figure 1). Receptor

In This Issue

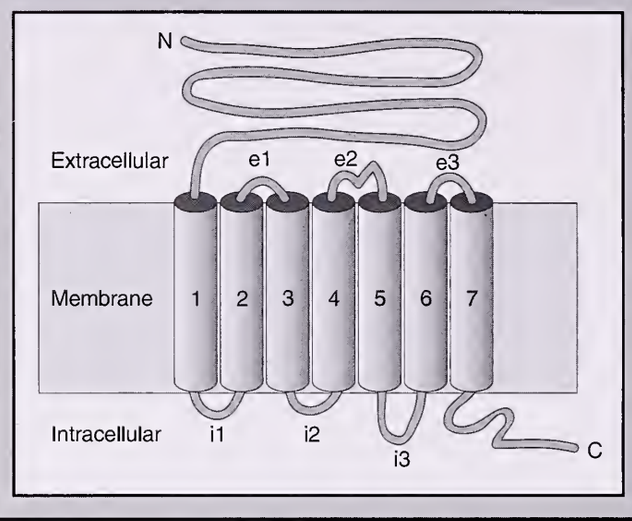
activation that accompanies agonist binding involves conformational changes in the transmembrane barrel that are relayed to the cytoplasmic surface. The concerted action of several intracellular loop regions, especially the N- and C-terminal ends of i3, has been implicated in G protein binding and activation.

Heterotrimeric G proteins are inactive in their guanosine diphosphate (GDP)-bound state, and interaction with an activated receptor is necessary to promote release of the nucleotide (Figure 2). Binding of ambient guanosine triphosphate (GTP) to the vacated site on the G protein α subunit leads to a change in the conformation of the G protein, dissociation of the complex, and effector activation by α and $\beta\gamma$ subunits. Once freed, the activated receptor can promote GDP dissociation from multiple other G protein molecules, thus providing signal amplification. Effector activation is terminated when the γ-phosphate of GTP is hydrolyzed by a guanosine triphosphatase (GTPase) that is intrinsic to the α subunit. Thus, GTP/GDP exchange is the rate-limiting step in the cycle, and GTPase serves as the critical timing mechanism. Different classes of G proteins are coupled to various effector mechanisms. The G protein that will be highlighted below is G_S, the G protein that stimulates adenylyl cyclase activity. The first disease shown to be due to a G protein defect was cholera, in which a bacterial toxin catalyzes adenosine diphosphate (ADP)-ribosylation of Arg201 in $G_S\alpha$ in intestinal cells. This covalent modification inhibits GTPase activity, leads to persistent production of cyclic adenosine monophosphate (cAMP) in the presence of little or no hormone, and causes severe secretory diarrhea.

ACTIVATING MUTATION OF G PROTEIN α SUBUNIT GENES IN TUMORS

The first example of an activating mutation in a G protein α subunit gene was the discovery of somatic heterozygous mutations of $G_S\alpha$ (*gsp* mutations) in a subset of growth hormone (GH)-secreting tumors of human pituitary.[5,6] One set of mutations involved Arg201 (the cholera toxin target) and another affected Gln227. Substitution of either of these amino acid residues in vitro has been shown to inhibit GTPase activity and lead to inappropriate stimulation of adenylyl cyclase. Recent crystallographic data show that these residues are located adjacent to the γ-phosphate of GTP, and it is now easy to see why changes at either of these critical positions would interfere with GTP hydrolysis.[7] Mutations of *gsp* were subsequently identified in some thyroid tumors.[8-10] The pathology associated with *gsp* mutations is consistent with the known effects of cAMP in mediating increased cell proliferation and secretion of hormone in somatotropes and thyroid follicular cells.[11]

Figure 1
Predicted Structure of a G Protein-Coupled Receptor



$G_S\alpha$ MUTATION IN McCUNE-ALBRIGHT SYNDROME

McCune-Albright syndrome (MAS) is a sporadic disease classically defined by polyostotic fibrous dysplasia, café au lait spots, sexual precocity, and other hyperfunctional endocrinopathies.[12,13] Endocrine tissues that function autonomously in MAS include the gonads, thyroid, adrenal cortex, and pituitary somatotropes. The sporadic occurrence of MAS, its variable presentation, and the distinctive pattern of skin pigmentation led Happle[14] to hypothesize that the disorder was due to a dominant somatic mutation occurring early in embryogenesis. According to this model, patients with MAS are mosaic for the mutant gene (Figure 3). Because cAMP was known to stimulate the growth or function of tissues classically involved in MAS, it was proposed that the mutant gene was one that led to excess cAMP production.[12,15]

In Future Issues

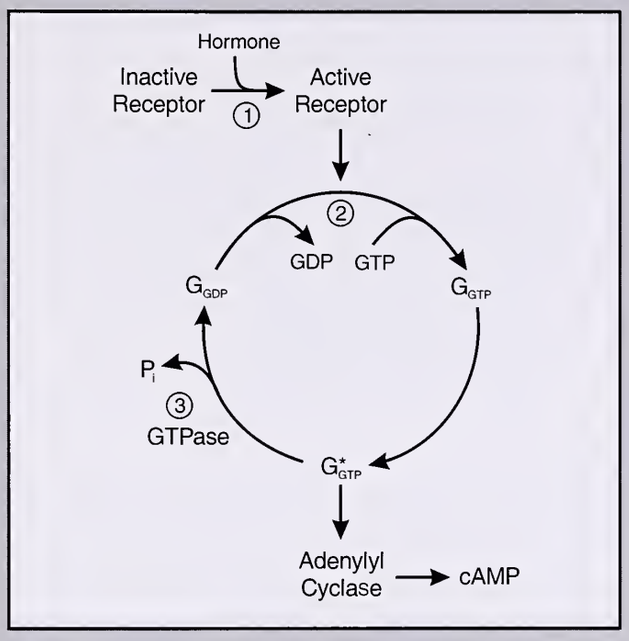
Genes of Growth Factors and Hormones: An Update
Victor McKusick, MD

The Neuroendocrinology of Stress: Its Relationship to Growth
George Chrousos, MD

Indications for Leg-Lengthening Procedures and Current Status of the Technique
Deborah Stanitski, MD

Growth Hormone Treatment in Chronic Renal Insufficiency: An Update
Richard Fine, MD

Figure 2
The G Protein Cycle Coupled to Adenylyl Cyclase



Inappropriate stimulation of this pathway results from mutations that cause 1) receptor activation in the absence of hormone, 2) GTP/GDP exchange in the absence of activated receptor, or 3) inhibition of GTPase activity.

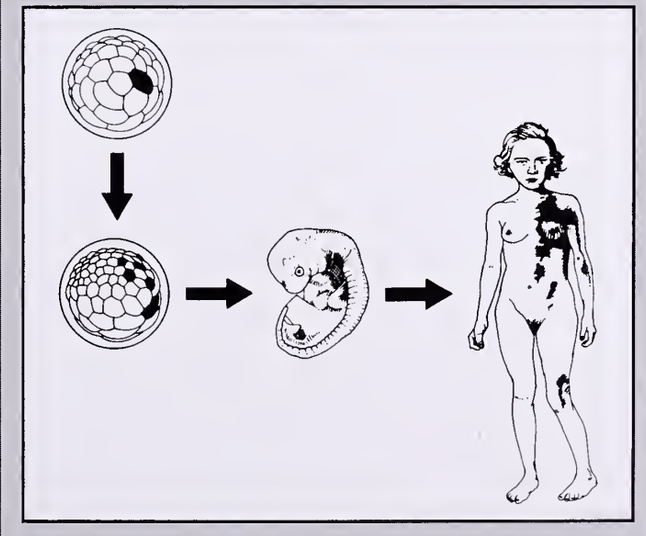
With the discovery of its role in isolated somatotrope tumors, $G_s\alpha$ became an excellent candidate gene for MAS, and mutations encoding substitution of Arg201 with either Cys or His were soon found in affected tissues from many MAS patients.[16-19] Mutant alleles were detected in variable abundance in different affected tissues from the same patient, including abnormal nonendocrine tissues, consistent with Happle's somatic mosaic model. Some types of cells harboring the $G_s\alpha$ mutation have increased proliferation, but others may have impaired growth. For example, even severely affected MAS patients typically have little or no evidence of mutation in DNA prepared from blood leukocytes, a fact that precludes straightforward molecular diagnosis. In only one case in which affected tissue was available for analysis has there been a failure to find the Arg201 mutation.[20] Mutations of Gln227 have not been found in MAS, possibly because it has a more powerfully activating, lethal effect on embryonic cells even when expressed in the mosaic state. Although most MAS patients manifest only classic features of the syndrome, a subset of patients with hepatobiliary abnormalities, cardiovascular disease, and early death has been described.[18]

Severe disease may be associated with an earlier mutational event that leads to a widespread distribution of mutant cells in the embryo, while incomplete forms of MAS may result from a mutational event that occurs later in embryologic development. A focal somatic mutation of $G_s\alpha$ gene during adulthood serves as the basis of diseases that affect only a single tissue, such as somatotrope or thyroid adenomas[5,8-10] and monostotic fibrous dysplasia.[21] Studying the pathophysiologic consequences of constitutive activation of G_s in bone and other MAS tissues may provide insight into the role that G_s normally plays in cellular development and differentiated function. Understanding the aberrant behavior of the mutant cells that produce lesions of fibrous dysplasia is certainly needed to develop better therapy for this painful and disabling condition.

OTHER ACTIVATING G PROTEIN MUTATIONS

Mutations that block GTPase activity of the α subunit of G_{i2} have been described in a very small number of ovarian and adrenocortical tumors,[8] and their general significance remains to be proven. A rare condition characterized by a combination of gonadotropin-independent male precocious puberty (gain of function) and pseudohypoparathyroidism type Ia (loss of function) has been shown to be due to a unique mutation of $G_s\alpha$ that allows spontaneous release of GDP in the absence of receptor (Figure 2).[22] At testis temperature (33°C), the mutant protein is constitutively active; however, at normal body temperature (37°C), it is rapidly degraded, thus explaining the paradoxical phenotype.

Figure 3
Somatic Mutation in Early Embryogenesis Produces Mosaicism in McCune-Albright Syndrome



Adapted from an illustration by Frank Netter. In: Netter FH. *The Endocrine System.* Vol 4. Summit, NJ: CIBA, 1965.

ACTIVATING RECEPTOR MUTATIONS

In the course of studying the structure of adrenergic receptors, it was observed[23] that substitution of a single Ala residue located at the junction of i3 and TM6 promoted activation of the receptor even in the absence of agonist. Furthermore, the mutant receptor was oncogenic when expressed in rodent fibroblasts.[24] The discovery that artificial mutagenesis could be used to generate receptors with unregulated activity raised the possibility that a naturally occurring gene mutation that led to constitutive activation of a GPCR could serve as a mechanism of human disease.

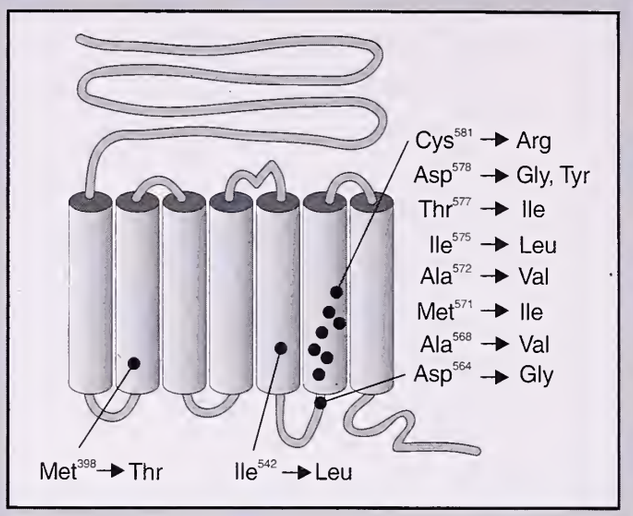
ACTIVATING MUTATIONS OF THE LUTEINIZING HORMONE RECEPTOR IN TESTOTOXICOSIS

Testotoxicosis, also known as familial male precocious puberty, is a gonadotropin-independent disorder that is inherited in an autosomal dominant, male-limited pattern.[25] Testosterone secretion and Leydig cell hyperplasia occur in the context of prepubertal levels of luteinizing hormone (LH), and the onset of puberty in affected boys usually occurs by 4 years of age. It was hypothesized that testotoxicosis was due to a mutant LH receptor (LHR) that could be activated in the presence of little or no agonist, and a heterozygous mutation that results in substitution of Asp578 in TM6 with Gly was first found in affected individuals from 9 different kindreds.[26,27]

To assess the functional effect of the Asp578→Gly mutation, wild-type and mutated human LHR were transiently expressed in COS-7 cells.[26] In contrast to the silent wild-type LHR, the mutant LHR produces a 4.5-fold increase in basal cAMP production in COS-7 cells, indicating that it is constitutively active. Agonist-independent stimulation of cAMP production represents about 40% of the maximal stimulation produced by the agonist human chorionic gonadotropin (hCG), and is not simply due to increased receptor expression.[28,29] The mutant receptor is also capable of responding to increasing concentrations of hCG, with a median effective concentration (EC_{50}) and maximal hCG-stimulated cAMP production similar to that of the wild-type receptor. The mutation has no effect on agonist binding affinity, which is known to be determined primarily by sequences in the large N-terminal domain.[28,29]

The Asp578→Gly LHR mutation is the most common cause of familial testotoxicosis, and it has also been detected in sporadic cases of gonadotropin-independent, male precocious puberty.[28,30] Different mutations of the LHR, mostly clustered in TM6, have been found in other patients.[27,29-33] The location of activating LHR mutations is shown in Figure 4.

Figure 4
Activating Mutations of the Luteinizing Hormone Receptor in Testotoxicosis



Some LHR mutations produce biochemical phenotypes similar to that of the Asp578→Gly substitution, but others do not. For example, an LHR mutation encoding substitution of Asp578 with Tyr promotes much higher basal cAMP accumulation in transfected cells than that produced by the other mutations.[30,34] This mutation has been found in 3 unrelated boys with unusually early signs of puberty, suggesting that their clinical phenotype is related to the strongly activating nature of the Asp578→Tyr substitution.

Dominant mutations that lead to constitutive activation of the LHR-mediated cAMP signaling pathway can explain the pathophysiology of gonadotropin-independent precocious puberty in males. LHR-mediated effects, including testosterone production, are known to involve increased production of cellular cAMP.[35] Intracellular cAMP accumulation triggered by unoccupied mutant receptors appears sufficient to cause Leydig cell hyperfunction and hyperplasia, although the delay in phenotypic expression must be related to other developmental events. LH alone is adequate to trigger steroidogenesis in Leydig cells, but both LH and follicle-stimulating hormone (FSH) are required to activate ovarian steroidogenesis, a fact that explains why females carrying the mutant allele do not exhibit precocious puberty.

In contrast to G proteins, the actual 3-dimensional structure of a GPCR has not yet been defined. Activating LHR mutations may provide insight into structural features involved in receptor activation. The location of most of the mutations is consistent with earlier data, which show that residues at the base of TM6 and in the adjacent C-terminal portion of i3 play a critical role in G protein coupling. In the inactive receptor state, the conformation of TM6

may be restricted by a set of interhelical bonds. One can imagine that hormone binding serves to break these constraints, thus allowing key residues on the cytoplasmic face to become exposed. Substitution of certain residues in TM6 may partially mimic agonist occupancy by weakening or eliminating interhelical bonds. Although some GPCR substitutions appear to act by increasing the proportion of receptors in the active conformation, it is possible that other activating substitutions will be found to act primarily by increasing the affinity of the isomerized receptor for G protein or by interfering with normal desensitization mechanisms.

ACTIVATING RECEPTOR MUTATIONS IN OTHER DISEASES

Knowledge that the growth and function of thyroid follicular cells are positively regulated by cAMP inspired the successful search for activating thyroid-stimulating hormone receptor (TSHR) mutations in hyperfunctional thyroid adenomas. Several different somatic TSHR gene mutations have been identified in sporadic adenomas, and autosomal dominant thyroid hyperplasia has been shown to represent the result of germline mutations in the TSHR gene.[10,36-38] As with LHR mutations in testotoxicosis, many of the TSHR mutations are clustered in TM6, but substitutions found in e1, e2, TM3, and TM7 indicate that other regions of the TSHR must also participate in stabilizing the inactive receptor conformation.

The parathyroid and kidney help maintain extracellular Ca^{2+} concentrations within a narrow physiologic range by promptly responding to increased Ca^{2+} with decreased parathyroid hormone (PTH) secretion and decreased Ca^{2+} reabsorption, respectively. This process has recently been shown to be mediated by a G protein-coupled Ca^{2+} receptor. Heterozygous mutations encoding 2 different substitutions in this receptor have been found in affected members of 2 kindreds with autosomal dominant hypocalcemia, indicating that increased receptor sensitivity to extracellular Ca^{2+} or constitutive receptor activity is responsible for this rare disorder.[39,40]

Jansen's disease, or metaphyseal chondrodysplasia, is an uncommon form of dwarfism often associated with PTH-independent hypercalcemia and hypophosphatemia. It has been shown to be due to constitutively activating mutations of the PTH receptor that lead to abnormal formation of endochondral bone and inappropriate signaling in the kidney.[41,42]

FUTURE PROSPECTS

Therapy for patients with activating mutations of $G_s\alpha$ or $G_s\alpha$-coupled receptors is generally directed at blocking the downstream effects wrought by the overactive cAMP signaling cascade or ablation of the abnormally functioning tissue. Because somatotrope adenomas contain somatostatin receptors linked to inhibition of adenylyl cyclase activity, treatment with a somatostatin analogue can be beneficial. The realization that some types of receptor antagonists have the ability to preferentially bind and stabilize the inactive conformation of a receptor (so-called negative antagonists) raises the possibility that such drugs could someday be used to treat diseases due to agonist-independent receptor activity.[43]

Is it possible to predict other diseases that might be due to activating mutations in G protein-coupled pathways? In several of the syndromes discussed above, hypotheses guided by knowledge of the biochemistry, pathophysiology, and genetics of a disease have led to the discovery of the causative gene mutations; however, other searches have been less fruitful.[44,45] It has been suggested that activated GPCR and $G\alpha$ subunit genes with proven oncogenic potential in cultured rodent fibroblasts[24,46] might be detected in some types of human malignancy. The phenotypes of transgenic mice expressing activated receptors or G protein subunits may also provide clues to human disease. Finally, it is important to consider that variation in the genes that encode other components in the G protein signaling cascade, including the β and γ subunits, effectors, and proteins involved in receptor desensitization, may also be found to play a role in some forms of endocrine disease.

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From the Endocrine Society Meeting, June 13, 1996

GH Axis – Child and Adolescent: A Review of the Clinical Oral Session

Eight papers were presented. The first was entitled, *IUGR and Postnatal Growth Failure in a Patient Homozygous for a Partial IGF-1 Gene Deletion* (OR 46-1).[1] The first case of a partial insulin-like growth factor 1 (IGF-1) gene deletion was described in a human. The patient was a 15-year-old boy with a birth weight of 1.37 kg at 37 weeks gestation and a height standard deviation score (SDS) of -6.9 at 15 years of age. He was resistant to exogenous growth hormone (GH), had sensorineural deafness and moderate mental retardation. Serum GH was elevated, and IGF-1 was practically nonexistent. Partial deletion of the IGF-1 gene is compatible with life. IGF-1 is important in prenatal and postnatal growth and possibly in central nervous system development.

The second was entitled, *Dwarfism of Sindh: A Novel Form of Familial Isolated GHD Linked to the Locus for GHRHR* (OR 46-2).[2] Eighteen dwarfs in Pakistan inherited an autosomal recessive GH-releasing hormone receptor (GHRHR) defect, with phenotypes resembling GH deficiency (GHD) or GH insensitivity. GH, IGF-1, IGF-binding protein-3 were low and failed to increase with GHRH or other pharmacologic stimuli for GH release. An inactivating mutation in the GHRHR appears likely on the basis of LOD scores.

The third paper (OR 46-3) dealt with the correlation of hormonal circadian rhythms with types 1 and 3 procollagens. PICP (type 1) increased markedly, following GH pulsations, and decreased markedly following cortisol elevation.[3] PHINP (type III) did not change. The findings suggested that PICP levels in the morning may be low due to morning cortisol elevation, and time standardization is important when evaluating this test. By inference, interpretation of PICP levels may be hazardous because of marked fluctuations over *brief* periods.

The fourth paper (OR 46-4) dealt with intranasal use of GHR peptide as a therapeutic agent. GHR peptide in short, GH-sufficient children produced a modest average increase of 2.1 cm/y during 9 to 10 months of treatment.[4] The emphasis was not on growth, but a fall in the GH released over time to intranasal hexarelin, which occurred without a fall in the initial IGF-1 increased levels. The effective intranasal dose for GH release was 20 times the intravenous dose required. Hexarelin was given three times daily.

The fifth presentation was entitled, *Contrasted Doses of GH to GHD Patients in Respect to Achieving Respectable Adult Heights* (OR 46-5).[5] Doses of 0.06 to 0.19 mg/kg/wk given to GHD patients with spontaneous puberty produced no gain in height SDS during puberty. Doses of 0.3 mg/kg/wk continued through puberty advanced the mean SDS from -2.1 ± 1.4 at initiation of puberty to 0.9 ± 1.2 at completion of puberty. The larger dose more closely simulates the secretion rate of

GH during puberty, when GH release in the normal adolescent is stimulated by sex steroid secretion.

The sixth presentation was entitled, *Catch up Growth and Height Achievement in Older, Late-Treated GHD Patients* (OR 46-6). It pertained to GHD children >15 years of age who were minimally or not sexually developed when treatment was instituted.[6] Their mean bone age was 12.2 ± 1.8 years. Year 1, 2, and 3 growth rates with treatment were 8.5 ± 3.1 cm, 7.2 ± 2.3 cm, and 6.0 ± 2.0 cm, respectively. The conclusions were that over the 3 years, improvement of height age (3.2 ± 1.2 years), height SDS (2.3 ± 1.1 SD), and Bayley-Pinneau predicted height (0.9 ± 1.4 SD) were observed. Two patients over 20 years of age, who were sexually infantile, responded similarly.

The seventh paper was from a collaborative European study, entitled *Four Years of GH Therapy in 3 Dosage Regimens in 216 Children With ISS* (OR 46-7).[7] The conclusions were that GH at 3.0 or 4.5 IU/m^2 (1.0 to 1.5 mg/m^2) resulted in a doubling of the height velocity during the first year. Increasing the dosage after the first year (3.0 to 4.5 IU/m^2) reduced the waning growth effect. Growth and final height prognosis improved during 4 years of GH therapy. This was better with 4.5 IU/m^2 than with 3 IU/m^2.

The eighth presentation, a report from European collaborators, dealt with long-term response to rhGH treatment in Turner syndrome (OR 46-8). One hundred ninety patients were studied. The Europeans concluded that a 5.0-cm increment (corrected) was added with GH treatment, with wide individual variation. A significant discussion ensued regarding the effect and necessity of beginning GH therapy earlier than the ≥9 years of age (average) for patients in the reported study.

Robert M. Blizzard, MD

1. Woods KA, et al. Department of Endocrinology, St. Bartholomew's Hospital Medical College, London.
2. Maheshwari H, et al. Department of Medicine & Pediatrics, Northwestern University Medical School, Chicago.
3. Fiallo RV, et al. Department of Pediatrics, New York Hospital–Cornell Medical Center, New York.
4. Laron Z, et al. Department of Pediatric Endocrinology, Tel Aviv University.
5. Blethen SL, et al. Department of Pediatrics, SUNY, Stony Brook, New York.
6. Brasel JA, et al. Harbor UCLA Medical Center, Torrance, California.
7. Mombarg LTM, et al. Department of Pediatrics, University Hospital, Leiden.
8. Van der Boeck J, et al. Department of Pediatrics, Academic Hospital, Leiden.

Abstracts From the Literature

Short Stature Caused by a Mutant Growth Hormone

The authors studied a 4.9-year-old boy with short stature (height,-6.1 SD below mean for age and sex) whose growth was normal in utero. Basal and stimulated secretion of immunoreactive growth hormone (GH) was normal but levels of bioactive GH were subnormal. He responded to the administration of GH with an increase in growth rate. Isoelectric focusing was performed, and 2 GH peaks were detected in the proband in comparison to 1 peak in normal subjects. Examination of the *GH-1* gene revealed a heterozygous mutation (guanine to cytosine transversion) of 1 GH gene allele at codon 77 in exon 4, with substitution of cysteine for arginine. This mutation is near a controlling point for the binding of GH to its receptor. Thus, the patient had 2 species of GH, 1 wild-type and 1 mutated form. A similar heterozygous mutation was found in the father, who was of normal height but who had 1 serum GH peak by isoelectric focusing. Further analysis of the mutated GH expressed in *Escherichia coli* revealed that it had normal immunoreactivity compared with wild-type GH, but that it bound to the extracellular domain of the GH receptor (ie, the GH-binding protein) 6-fold more avidly than did native GH. Since this mutated GH did not stimulate intracellular signaling pathways in IM-9 cells, which have GH receptors, it inhibited the biologic effects of wild-type GH in this system. The investigators suggest that the mutated form of GH impaired growth by antagonizing the effects of the native GH molecule, which was also synthesized and secreted by the patient. The reason why the father with the same heterozygous mutation in the *GH-1* gene did not express this abnormal allele was unexplained.

Takahashi Y, et al. *N Engl J Med* 1996;334:432-436.

Editor's comment: *In the last few months there has been great interest in children with idiopathic short stature (ISS). Partial GH insensitivity among patients with ISS was described earlier (*J Pediatr *1995;127:244-250, published in abstract form in* GGH *Vol 11[4]:8). This was followed by the description of specific mutations of the GH receptor gene associated with ISS (*N Engl J Med *1995;333:1093-1098, published in abstract form in* GGH *Vol 12[1]:14 & 15). Now, Takahashi et al describe a mutation in the GH gene itself that produced an abnormal GH and was clinically associated with short stature in the affected individual.*

These papers provide data heralding a new subset of patients in whom GH gene mutations or GH receptor abnormalities explain the bioinactivity of GH. The prevalence of these abnormalities in children with ISS is unknown. Moreover, the features that clinicians should follow to identify GH insensitivity or bioinactivity also are not clarified. The diagnosis of these conditions continues to be based upon esoteric, highly sophisticated biochemical assessments.

Fima Lifshitz, MD

2nd Editor's comment: *Long sought but heretofore undiscovered, the elusive bioinactive GH molecule has now been identified in 1 patient. Previously, many patients have been suspected of having a biologically inactive, but immunologically active, GH (Kowarski et al, 1978; Valenti et al, 1985; Hayek et al, 1978; Bright et al, 1983; and others). However, with the technology of the 1970s and 1980s, it was not possible to prove that such a syndrome exists. Isoelectric focusing, studies of gene structure, and the interest and expertise of these authors have made the suspected syndrome a fact. They clearly demonstrated that the proband synthesized and secreted an atypical form of GH that was able to bind with high affinity to the extracellular domain of the GH receptor, was unable to initiate signal transduction, and inhibited the biologic effects of native GH. The failure of the father with the same heterozygous mutation to express this phenotype demands further consideration.*

Allen W. Root, MD

Effects of Recombinant Human Growth Hormone (rhGH) Treatment in Intrauterine Growth-Retarded Preterm Newborn Infants on Growth, Body Composition and Energy Expenditure

The investigators from Amsterdam administered rhGH (1.0 IU/kg or 0.33 mg/kg/d) to 7 preterm infants (mean gestational age, 30.4 weeks) with IUGR (mean birth weight, 938 g) beginning at 7 days of age and continuing until achieved weight was 2,000 g (34 to 68 days of treatment). When compared with an untreated control group of IUGR preterm infants, there was no effect of rhGH on: time to doubling of birthweight; increments in body weight, length, and head circumference; ponderal index; serum glucose or insulin values; skin-fold thicknesses; total body water; or energy expenditure. The authors concluded that administration of rhGH had no effect on growth or energy metabolism in preterm infants with IUGR.

van Toledo-Eppinga L, et al. *Acta Paediatr* 1996;85:476-481.

Editor's comment: *Despite some problems with the applicability to IUGR preterm infants of the utilized methodology[1], the data are of interest because they indicate that even exceedingly high doses of rhGH cannot positively affect the growth or metabolism of such children. Whether such therapy can have adverse effects is unknown at present. The findings also indicate the need to search for growth factors other than rhGH (perhaps insulin-like growth factor 2, insulin, etc) that may be of benefit to IUGR preterm infants. The utility of rhGH in preterm infants with appropriate growth for gestational age has yet to be assessed.*

Allen W. Root, MD

1. Wollmann H, et al. *Acta Paediatr* 1996;85:398-400.

Prenatal Diagnosis of 45,X/46,XX Mosaicism and 45,X: Implications for Postnatal Outcome

Prenatal diagnosis of chromosomal abnormalities is available for families who have an option whether to continue the pregnancy. Twelve patients with 45,X/46,XX mosaicism were diagnosed prenatally by amniocentesis and subsequently evaluated at 3 months to 10 years of age. All have had normal linear growth. Four had anomalies, including esotropia and ptosis (1); labial fusion (1); atrial septal defect (1); and urogenital sinus, dysplastic kidneys, and hydrometrocolpos (1). The patient with ophthalmologic abnormalities is mentally delayed. None would have warranted karyotyping for clinical suspicion of Turner syndrome. These 12 were compared with 41 45,X/46,XX patients diagnosed postnatally. The prevalence of 45,X/46,XX mosaicism is 10-fold higher among amniocenteses than in series of postnatally diagnosed individuals with Turner Syndrome, which suggests that most individuals with this karyotype escape detection and that an ascertainment bias exists toward those with clinically evident abnormalities. The authors note that the phenomenon of a milder phenotype for the prenatal group is similar to that observed for 45,X/46,XY individuals diagnosed prenatally. The authors emphasize that prenatal counseling for 45,X/46,XX in the absence of such ultrasound abnormalities as hydrops fetalis should take into account the expectation of a milder phenotype than that of patients ascertained postnatally. The same does not hold true for 45,X diagnosed prenatally.

Koeberl DD, et al. *Am J Hum Genet* 1995;57:661-666.

Editor's comment: *This presentation provides important information for genetic counseling. One should not be too discouraging when discussing the expectations of a fetus when a 45,X/46,XX karyotype or a 45,X/46,XY karyotype is reported. Many of these 45,X/46,XX children will be phenotypically normal and possibly may end up with a 46,XX karyotype. The 45,X cell line may sometimes disappear, although that was not investigated in this study. This comment is made because the prevalence of 45,X/46,XX mosaicism is 10-fold higher among amniocenteses data than in series of postnatally diagnosed individuals with Turner syndrome.*

Judith G. Hall, MD

Behavioral Phenotypes in Dysmorphic Syndromes

Syndromes with congenital anomalies usually are diagnosed by their physical features or a particular combination of features that are observed on clinical grounds. Recently, objective means of defining and measuring behavior such as specific patterns of speech and language, types of attention deficits, particular social impairments, and other behavioral disturbances such as self-injury, skin scratching, and lip biting have been developed. These even have been quantified. Thus, it now becomes possible to define the specific behavior phenotypes in a number of syndromes. For instance, mimicking is common in Down syndrome. A discrepancy between performance and verbal skill also is typical of Turner syndrome. Impaired speech and language development often is found in Klinefelter syndrome. Learning and language difficulties, impaired social relations, and crimes against property often are found in individuals with the XYY syndrome. In the fragile X mental retardation syndrome, visuo-spatial skills are impaired such that there is difficulty in climbing stairs and problem solving for sequential events. Autistic and ritualistic disturbances also are frequent. In tuberous sclerosis, autism, hyperactivity, and hypsarrhythmic salaam attacks are seen. In Williams syndrome, superior vocal skills are observed, resulting in "cocktail party" chatter. Patients with this syndrome also have hyperacusis. In Prader-Willi syndrome, hypotonia, hyperphagia, and tantrums are typical; and in Angelman syndrome, a happy disposition with paroxysmal laughter and a jerky ataxic gait usually are seen. In Rett syndrome, loss of mental abilities together with hand-wringing are seen. In Sotos syndrome, hyperactivity, clumsiness, and poorly articulated speech are observed. The authors urge better description of behavior in future clinical reports.

Turk J, Hill P. *Clin Dysmorphol* 1995;4:105-115.

Editor's comment: *It is clear that defining the behavior seen in syndromes will help to make specific diagnoses. The opportunity to record movement and behavior using video cameras now exists. Just as with physical features, it is sometimes hard to describe accurately types of movements and various facial expressions. Recording and studying behavior will be important for the future in order to delineate the mechanisms involved in a particular disorder.*

Because of the lack of specificity and quantification in the past, it was often hard to describe the behavioral characteristics found in a specific syndrome. Abnormal respiration is another type of behavior, and is characteristic in the Joeebert syndrome. Our ability to define behavioral characteristics will increase with time. I expect there will be many "behavior" syndromes with normal physical features—after all, half the human genes have to do with the brain.

In addition, the authors have very thoroughly reviewed the historical and behavioral perspectives of various syndromes. Geneticists, pediatric endocrinologists, psychologists, and nurses dealing with syndromes should benefit significantly by reading the complete article.

Judith G. Hall, MD

Autoantibodies to the Extracellular Domain of the Calcium Sensing Receptor in Patients With Acquired Hypoparathyroidism

The autoimmune pathogenesis of acquired hypoparathyroidism has been difficult to document with certainty. Earlier studies reported the presence in sera from patients with acquired hypoparathyroidism of antibodies to parathyroid tissue identified by indirect immunofluorescence. Antibodies have also been observed that inhibit the secretion of parathyroid hormone, or that are cytotoxic to parathyroid cells, but such studies have been difficult to replicate. The present investigators hypothesized that patients with this disorder may have antibodies to the G protein-associated calcium sensing receptor, which is expressed on the cell membrane of parathyroid cells.

In preliminary studies, antibodies to extracts of human parathyroid glands were detected by immunoblot analysis in only 5 of 25 (20%) patients with acquired hypoparathyroidism. Since the antigen appeared to be of the same size as the calcium sensing receptor (120 to 140 kd), further studies utilizing the membrane calcium sensing receptor expressed in transfected cells were undertaken. In 8 of 25 (32%) patients (which included all those previously positive by immunoblot analysis of human parathyroid tissue), antibodies to this receptor were detected by immunoblot. When the calcium sensing receptor was differentially expressed as its extracellular domain and as its transmembrane-intracellular domain, 14 of 25 (56%) patients demonstrated antibodies to the extracellular portion of the receptor and none to the transmembrane-intracellular domain. Patients with both idiopathic acquired hypoparathyroidism as well as those with type I autoimmune polyglandular syndrome demonstrated antibodies to this segment of the calcium sensing receptor. In none of 50 patients with a variety of other autoimmune diseases or in normal controls were antibodies to this antigen detected.

The authors concluded that many patients with acquired hypoparathyroidism have antibodies to the extracellular portion of the calcium sensing receptor. They speculate that some patients in whom these antibodies were not detected may have had the disease for prolonged periods, leading to loss of the autoantigen needed for stimulation.

Li Y, et al. *J Clin Invest* 1996;97:910-914.

Editor's comment: *This report adds further data supporting the autoimmune etiology of acquired hypoparathyroidism in the majority of patients. The relationship of antibodies to the extracellular domain of the calcium sensing receptor in relation to the etiopathogenesis of acquired hypoparathyroidism is uncertain. In preliminary studies, the authors report that these antisera did not affect intracellular calcium levels in vitro in cells transfected with this receptor. These data indicate the need to study further the biologic activity of these antibodies and to search for other antigens that may be of pathophysiologic importance in this disorder.*

Allen W. Root, MD

2nd Editor's comment: *Exactly 30 years ago, Walter David, Darwin Chee, and I first reported the presence of parathyroid antibodies in the sera of hypoparathyroid patients (*Clin Exp Immunol *1966;1:119), as Li et al pointed out in their excellent article. Neufeld, Maclaren, and I then pursued over 15 years the theory that acquired hypoparathyroidism often was of autoimmune origin, but we and others had great difficulty in confirming our hypothesis in the laboratory. Li, Maclaren, and colleagues now have confirmed that autoantibodies exist against a specific component of the parathyroid cells. Observing this unraveling of questions and the near solving of the hypothesis over 30 years has been exciting and rewarding to me, and one of the pleasures and blessings of being given the opportunity to live and continue to be professionally active over such an extended period.*

Robert M. Blizzard, MD

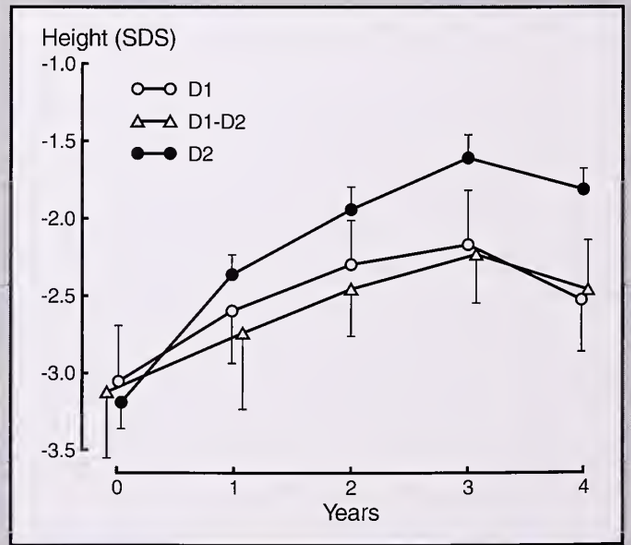
Follow-Up of Three Years of Treatment With Growth Hormone and of One Post-Treatment Year, in Children With Severe Growth Retardation of Intrauterine Onset

Job et al report follow-up data on their original randomized double-blind study of 2 doses of growth hormone (GH)—(0.4 IU/kg/wk (dose D1) or 1.2 IU/kg/wk (dose D2) (*J Clin Endocrinol Metab* 1994;78:1454-1460)—in prepubertal children with very short stature of intrauterine onset. Previously, they reported that growth velocity increased in intrauterine growth retarded (IUGR) children treated with GH in a dose-dependent manner. At the end of 2 years of GH treatment, subjects receiving the low dose of GH (D1) were randomized either to continue the same dose or be switched to the higher dose (D1D2) and treated for an additional year. Finally, a follow-up year of no GH treatment was added to their study. Seventy-eight subjects were studied. Both birth length and birth weight had to be -2 SD or more below the mean for gestational age; height at admission had to be -2 SD or more below the mean according to the usual French standards; bone age had to be either retarded or equal to the chronologic age; and the growth velocity for the previous 12 months could not exceed the mean for age. In addition, all patients had to be prepubertal. Height and sexual development were assessed every 3 months during GH treatment and every 6 months during the posttreatment year at 10 different centers in France and Belgium. In addition to careful height and weight measurements and assessment of sexual development, bone age was determined by the method of Gruelich and Pyle every 6 months. Sixty-six children remained in the study at follow-up.

Average age at the onset of the study in 1988 was 8.1 ± 0.2 years. The mean annual height velocities were greatest during the initial year of GH treatment and subsequently declined. At the end of 3 years of treatment, the height reached -2.37 SD in D1, -2.17 in D1D2, and -1.58 in D2 (Figure 1). The total height gain was 0.77 ± 0.1 SD in D1, 0.93 ± 0.15 SD in D1D2, and 1.61 ± 0.08 SD in D2. The percentage of children whose height was within the normal range for age was 46.7% in D1, 52.2% in D1D2, and 70% in D2.

During the follow-up year without treatment, growth deceleration was observed in most patients, with mean growth velocity falling below -1 SD. The mean loss in height was approximately 0.25 SD for age. Skeletal maturation over 36 months of GH treatment was not significantly different among the 3 groups. Mean bone age, however, remained retarded in all 3 groups at the end of the fourth year of study. There were no significant differences among the 3 groups in the frequency of occurrence of puberty or in age at its onset; the rate of sexual maturation after its onset did not differ among the groups.

Figure 1



Mean (± SEM) height, expressed as SD for age at annual visits. The data are generated from the same patients over 4 years (D1, *n* = 11; DID2, *n* = 17; D2, *n* = 29) followed longitudinally.

The authors state that their data confirm that GH treatment can accelerate the growth of IUGR short children beyond 2 years of treatment despite "the waning effect" of GH and that this growth is accompanied by some degree of acceleration in bone maturation. The authors note that the strengths of their study include: (1) the cohort, which excluded familial short stature but did include 6 cases of Silver-Russell–type dwarfism, was homogeneous; (2) puberty began within the normal age range; and (3) they included the growth velocity after discontinuation of GH treatment. There are no data, however, on final heights.

Job JC, et al. *Pediatr Res* 1996;39:354-359.

Editor's comment: *This is a very interesting paper. Job et al have performed an evaluation of long-term use of GH in IUGR short children. It would be of interest to have more information with regard to the range of bone age retardation in the patients when initially seen. With mean heights at the end of the study averaging from -1.8 ± 0.2 SD to -2.5 ± 0.4 SD and bone age being delayed approximately 1 year or more, it is unclear whether a significant number of these children also have constitutional delay of growth and adolescence. In addition, the inclusion of children with Russell-Silver syndrome may have adversely affected the growth response data. However, the authors are to be congratulated in carrying out such a long-term study and including a year of follow-up. It would have been interesting to have included a control population of similarly height-challenged IUGR patients who were not treated and were of similar age. We would hope Job and colleagues will continue their studies and report final heights in these patients in the next few years.*

William L. Clarke, MD

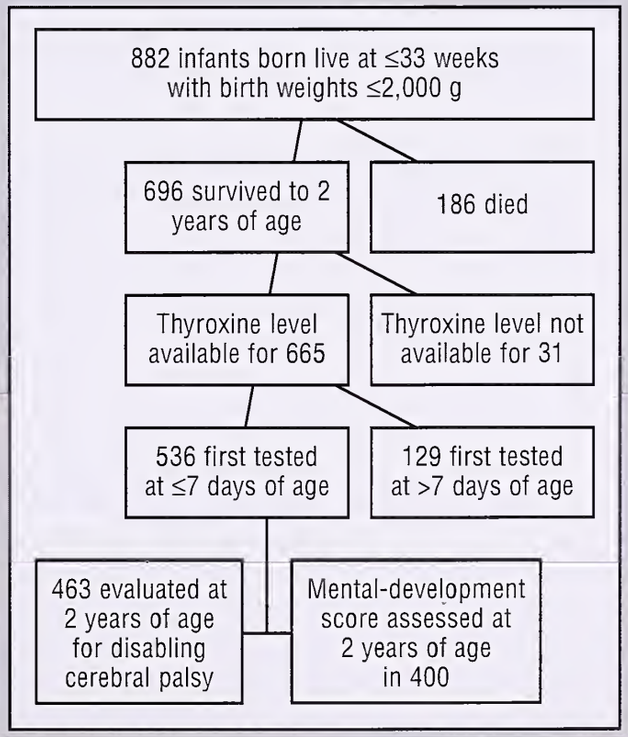
The Relation of Transient Hypothyroxinemia in Preterm Infants to Neurologic Development at Two Years of Age

Taking advantage of the prospective design of the Central New Jersey Neonatal Brain Hemorrhage Study, this retrospective study was performed in a historical cohort. The authors chose those infants who were born at 33 weeks of gestation or earlier, who had undergone screening for congenital hypothyroidism within the first 7 days of life, and who survived until the age of 2 years or beyond (n=536; Figure 1). The levels of thyroxine were retrieved from the newborn screening program and were expressed as a SD score (SDS) to correct for the daily interassay variation. Severe hypothyroxinemia was defined as a blood thyroxine value more than 2.6 SD below the mean for New Jersey newborns. None of the infants had congenital hypothyroidism.

Neurologic and developmental outcomes were assessed at 2 years of age by means of the Bayley Psychomotor Developmental Index and the Bayley Mental Developmental Index or the Stanford-Binet Intelligence Scales for Children. Emphasis was placed on the presence of disabling cerebral palsy and/or low mental developmental scores. Twenty-two prenatal, perinatal, and early neonatal variables were analyzed in order to adjust for any association between hypothyroxinemia and a given neurodevelopmental outcome. Infants with severe hypothyroxinemia had a risk of disabling cerebral palsy that, depending on the extent of adjustment for covariates, was 4.4 to 17.6 times that of the infants with normal thyroxine concentrations. The mental development scores at 2 years of age were 8 to 18 points lower in infants who had had severe hypothyroxinemia than in those with normal thyroxine levels. The authors conclude that severe hypothyroxinemia in preterm infants may be an important cause of problems in neurologic and mental development detected by 2 years of age.

Reuss ML, et al. *N Engl J Med* 1996;334:821-827.

Figure 1
Enrollment and Assessment of the Study Subjects



The study enrolled 882 infants born at or before 33 weeks of gestation who were therefore at risk for hypothyroxinemia. The infants were drawn from the 1,105 newborns with birth weights of 2,000 g or less who were enrolled in the population-based Central New Jersey Neonatal Brain Hemorrhage Study.

Editor's comment: *This paper is very important as it provides data indicating that transient hypothyroxinemia without hyperthyrotropinemia in preterm infants is not benign. The study by Reuss et al rings a bell of alarm and prompts us to approach these infants more carefully. The mechanism of transient hypothyroxinemia in preterm infants, however, has not been elucidated. It may involve either a metabolic adaptation to nonthyroidal illness or an incomplete maturation of the hypothalamic-pituitary-thyroid axis as discussed by Vulsma and Kok[1] in the editorial comments that accompanied the paper.*

The traditional belief that the fetus does not need thyroxine for intrauterine development was based on the assumption that negligible amounts of thyroxine from the mother crossed the placenta. This belief was first challenged when significant passage of thyroxine from the mother to the fetus was identified, and it is now being challenged again with the findings of Reuss et al of poor mental and developmental outcomes of preterm infants displaying transient hypothyroxinemia. Treatment of these infants aiming to correct the subnormal levels of thyroxine as soon as detected will be the next step, but this should be undertaken only in a controlled study.

Fima Lifshitz, MD

1. Vulsma T, Kok JH. *N Engl J Med* 1996;334:857-858.

2nd Editor's comment: *Having just reviewed this abstract and Dr. Lifshitz's editorial comment, I attended The Endocrine Society meeting in San Francisco and read an abstract by Dr. M.K. Hunter et al entitled, (Program, 10th IC of Endocrinology, Vol II: June 14 and 15, 1996, OR 48-4, page 7113).* Follow-up of Newborns With Low T^4 and Non-Elevated TSH Concentrations. *The content of the abstract was related to the article by Reuss et al. Therefore, the important comments and data follow:*

Over a 20-year period, the Northwest Regional Screening Program screened 1,747,805 newborn infants. Follow-up of infants with low thyroxine levels without thyrotropin (TSH) elevation led to the diagnosis of hypothyroidism in 60, including 25 infants with delay in TSH rise (1:67,226 infants), 9 infants with mild hypothyroidism (TSH <25 IU/L), and 26 infants with hypopituitary hypothyroidism (1:67,223), in addition to 4,334 infants with thyroid-binding globulin deficiency (1:4,027). Follow-up was scheduled at 1 year of age.

These data indicate that follow-up of infants (preterm or term) with low thyroxine and normal TSH levels is important.

Robert M. Blizzard, MD

Insulin-Like Growth Factor Binding Protein-3 Generation: An Index of Growth Hormone (GH) Insensitivity

Eleven children with possible growth hormone (GH) insensitivity (GHI) and 8 children with proven GH deficiency (GHD) were studied with an insulin-like growth factor (IGF) generation test in which IGF-1, IGFBP-3, and GHBP were measured before starting a 4-day course of subcutaneous GH at 0.1 U/kg/d, and 12 hours after the last GH injection. GHI was defined based on short stature for target height (-2 SD for midparental height), a high basal GH (>10 mU/L), and/or high peak GH (>40 mU/L) on a standard GH provocation test. GHD was defined based on a peak GH response to arginine ≤10 mU/L. The 2 groups were comparable in terms of their age, body mass index, height, and growth velocity. The change in these parameters was analyzed as an absolute increment, as an increment in SDS, and as a percentage change. None of the children fulfilled the Pharmacia Study Group IGF generation test criteria for the diagnosis of Laron syndrome; ie, IGF-1 increment <15 µg/L and IGFBP-3 increment <0.4 mg/L. The results of ΔIGF-1 and ΔGHBP in the generation test did not show statistical differences (ie, could not discriminate) between the GHI and the GHD patients regardless of whether the results were analyzed as absolute change, as percentage increment, or SDS increment. However, the results of IGFBP-3 showed statistical differences between the 2 groups of patients when comparing the poststimulation peaks by increment as well as the percentage increments. Significant *inverse* correlations were found between peak GH obtained during provocative tests. Both the IGF-1 and IGFBP-3 rose in the IGF generation test. Percentage increase of IGFBP-3 was identified as the most significant parameter to predict GH peak by stepwise multiple regression analysis.

The authors speculate that some children may have selective resistance in either the GH–IGF-1 axis or the GH–IGFBP-3 axis, given the varied combination of responses found in the study. IGF-1 generation per se was inadequate as an index of partial GHI and should be used in conjunction with IGFBP-3 generation.

Thalange NKS, et al. *Pediatr Res* 1996;39:849-855

Editor's comment: *Bioinactive GH secondary to mutations of the GH gene[1] and GH receptor mutations associated with clinical pictures of partial GHI[2,3] (see* GGH *vols 11:4 and 12:1, respectively) have been recently identified. Although these newly described and documented entities definitely are helping us understand the different pathophysiologic mechanisms of short stature, accurate diagnosis can be made only with sophisticated technology. Their clinical recognition continues to be elusive. This paper by Thalange et al attempts to identify easily available biochemical markers in the context of dynamic testing, which may yield a diagnostic clue in identifying patients*

with GHI. Although their results support the IGFBP-3 generation test as a suitable tool to include in the diagnostic approach, its degree of uncertainty is still considerable. The number of patients included in this report is small, and much heterogeneity was found in the basal levels of both IGF-1 and IGFBP-3. Selective blocks of IGF-1 versus IGFBP-3 generations have been proposed to explain some differences. This concept permits the suggestion that post-receptor defects currently unexplored may constitute another etiologic category of short children identified as having idiopathic short stature.

Fima Lifshitz, MD

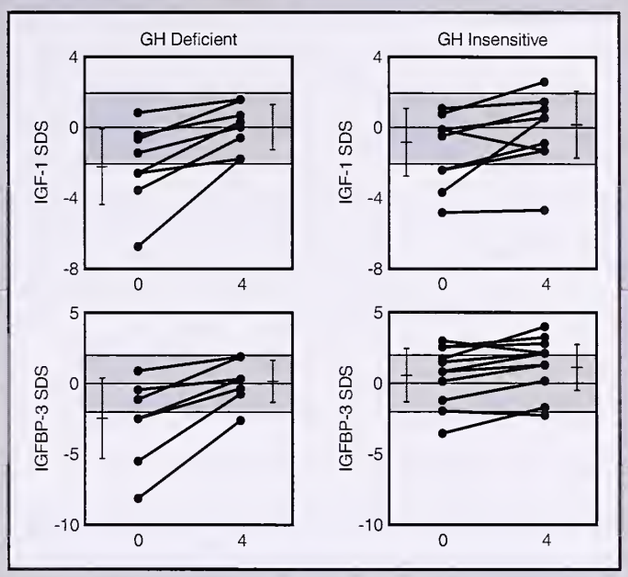
1. Takahashi Y, et al. *N Engl J Med* 1996;334:432-436.
2. Attie KM, et al. *J Pediatr* 1995;127:244-250.
3. Goddard AD, et al. *N Engl J Med* 1995;333:1093-1098.

2nd Editor's comment: *The authors are reputable investigators who have attempted to clarify the confusion that exists about GHI. As so often in the past when trying to elucidate the presence of partial GHD, their work has not provided us with a tool to clinically detect partial GHI. For one or many reasons, a majority of the GHD and GHI patients did not have expected biochemical baseline results, and the responses to rhGH were very variable (Figure 1). This study would have been enhanced if the purported GHI individuals had been limited to nondysmorphic, idiopathically short persons (4 of the alleged GHI patients had syndromes); if bone ages had been included in the auxologic data to permit readers to better formulate their own impressions regarding the inclusion of short children in the GHI category; and if the patients with sexual development were specified with respect to their stage of pubertal development. Regardless of the deficiencies in the study and/or presentation, the authors are to be commended for their attempt to solve a complex problem: how to diagnose partial GHI.*

The authors have been invited to respond to these remarks, which are intended to be constructive, by writing a letter to the Editor of GROWTH, Genetics & Hormones *for publication in a future issue.*

Robert M. Blizzard, MD

Figure 1



Individual basal and peak IGF-1 and IGFBP-3 SDS in response to rhGH for GH-insensitive and GH-deficient subjects. Normal ranges for basal IGF-1 and IGFBP-3 are indicated by the shaded areas (± 2 SD).

Testicular and Ovarian Resistance to Luteinizing Hormone Caused by Inactivating Mutations of the Luteinizing Hormone Receptor Gene

The investigators report 2 families with different homozygous mutations of the luteinizing hormone receptor (LHR) gene. Both mutations lead to inactivation of the LHR but each produces a different phenotype. In the first family, 3 phenotypically female siblings, 15, 23, and 32 years of age, had XY karyotypes; absence of thelarche but normal pubarche; inguinal gonads; and absence of müllerian duct structures. A cytosine to thymine transition was identified at nucleotide 1660, leading to substitution at amino acid 554 of a stop codon (TGA) for arginine (CGA) within the third intracytoplasmic loop of the LHR. Thus, a truncated and nonfunctional LHR resulted. The same mutation was present in a 22-year-old XX sibling who had normal secondary sexual development, 1 episode of vaginal bleeding, and then prolonged amenorrhea. She had a small uterus, cystic ovaries, and elevated luteinizing hormone (LH) but normal follicle-stimulating hormone (FSH) levels.

In the second family, a male child with micropenis had a cytosine to adenine transversion at nucleotide 1847, leading to alteration of amino acid 616 from serine (TCT) to tyrosine (TAT) within the seventh transmembrane domain of the LHR. There was no testosterone secretory response to human chorionic gonadotropin, but normal adrenocortical response to corticotropin. Expression of the mutated form of the LHR in COS-7 cells revealed that it did not bind LH or transmit an intracellular signal.

Latronico AC, et al. *N Engl J Med* 1996;334:507-512.

Editor's comment: *The association of a homozygous inactivating mutation of the LHR with male pseudohermaphroditism has been anticipated and, indeed, previously reported[1] and abstracted in* GROWTH, Genetics & Hormones *1996;12(2):24.*

Of interest is the phenotype of an XX individual who is homozygous for the same mutation. This woman had secondary amenorrhea but no other obvious clinical manifestation of this mutation. This observation is instructive because it indicates that (1) a functional LHR is not necessary for pubertal ovarian function; (2) normal female puberty through menarche can be guided by FSH alone; (3) adrenal androgens alone are sufficient for normal sexual hair growth in the female (thus confirming other data); and (4) the heterozygous loss of 1 functional LHR is of no clinical or reproductive consequence. (The parents of the affected children were not studied but had 14 children.)

A mutation in the seventh transmembrane domain of the LHR in the child in family 2 prevents movement from the endoplasmic reticulum to the plasma membrane surface and thus binding of LH to its receptor. Since the affected subject with this defect had micropenis rather than ambiguous genitalia, presumably functional LHR was expressed on the fetal Leydig cell membrane in the first trimester of gestation, but not thereafter. (See GROWTH, Genetics & Hormones *1996;12[2]:24—2nd editor's comment.)*

Allen W. Root, MD

1. Kremer H, et al. *Nature Genet* 1995;9:160-164.

Teratogen Update: Diethylstilbestrol

Diethylstilbestrol (DES) teratogenicity occurred over a period of about 3 decades when it was used to avert miscarriage. Female fetuses who had a significant exposure to DES and other synthetic estrogens (now collectively referred to as DES) are now known to be at risk for carcinogenic and teratogenic effects. DES-exposed daughters have an increased risk for developing clear cell adenocarcinomas of the vagina and cervix and structural abnormalities of the genital tract that predispose to vaginal adenosis and other vaginal epithelial changes. Some male fetuses exposed to DES have structural abnormalities of the genital tract, but as yet no increase in cancer has been reported. Fertility and sexual function in these men appear to be normal. Girls exposed in utero to DES also have a somewhat higher risk of breast cancer than women who were not exposed. There is no evidence that grandchildren of DES-exposed daughters and sons have any abnormalities. It would appear that the epidemic of clear cell adenocarcinoma is over. It is not entirely clear whether there may be problems in intrauterine DES-exposed individuals who now are over the age of 50. Carcinomas developed in only a small proportion of this population. It appears that the mechanism by which DES caused these problems has to do with interfering with the "natural regression" of certain tissues in embryonic and fetal life.

Mittendorf R. Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) in utero. *Teratology* 1995;51:435-445.

Wilcox AJ, et al. Fertility in men exposed prenatally to diethylstilbestrol. *N Engl J Med* 1995;332:1411-1416.

Editor's comment: *These papers are helpful for reassuring at-risk individuals. The sad part of the whole DES story is that there was no beneficial effect in maintaining pregnancies and, consequently, a large number of children were exposed unnecessarily to DES. We must remind ourselves to be sure before prescribing a therapeutic agent that there is in fact a demonstrated therapeutic effect. We then must weigh the potential positive effect against the possible negative effects. Today we would like to think that clinical studies ensure that all therapies actually do what they are meant to do. However, possible long-term adverse effects are hard to predict, and a judicious approach to any therapy is obligatory under the Hippocratic oath to do no harm. Fortunately, future research funded by National Cancer Institute will permit monitoring of the DES-exposed population to determine whether any other abnormalities become apparent.*

Those who wish to read a very complete and extensive review of the DES story are referred to Mittendorf's article. Wilcox's article is more limited in scope, as it is confined to findings in males; nevertheless, it is an important report.

Judith G. Hall, MD

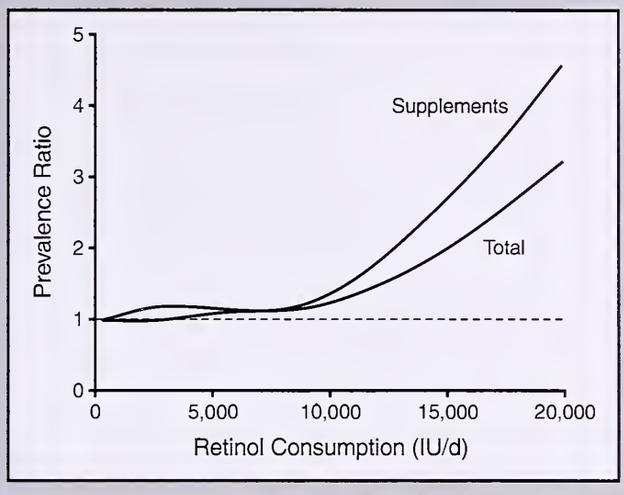
Teratogenicity of High Vitamin A Intake

In general, vitamins are thought to be essential for embryogenesis and necessary for health in the fetus, infant, child, and adult. However, fat-soluble vitamins have been recognized to cause toxicity and, potentially, teratogenicity when taken in large doses. Vitamin A is available in many forms as part of supplementary vitamin capsules. It also is present in the diet, coming from certain vegetables and animal sources, including dairy products, liver, and fortified foods. Currently, the recommended daily allowance of vitamin A for women is 800 retinol equivalents, which corresponds to 2,700 IU. Vitamin A has been found to be teratogenic in humans, and recently there has been an epidemic of teratogenicity because of isoretinoid used to treat severe acne. The malformations that can be seen in retinoic acid embryopathy include craniofacial, cardiac, thymic, and central nervous system abnormalities (Table 1).

Table 1
Birth Defects According to Category With Retinoic Acid Embryopathy

Type of Defect	No.
Cranial neural crest	
Craniofacial, central nervous system (except neural tube), and thymic	69
Heart	52
Total	121
Neural tube	48
Musculoskeletal and Urogenital	
Musculoskeletal	58
Urogenital	42
Total	100
Other	
Gastrointestinal	24
Nongastrointestinal	46
Total	70
Total	339

Figure 1
Retinol Consumption (IU/d)



Estimated prevalence ratio for birth defects related to the cranial neural crest, according to retinol intake during the first trimester of pregnancy.

Rothman et al have interviewed 22,748 women concerning their diet and illnesses during the first trimester of pregnancy. All sources of retinol intake were tabulated and an association was made with various types of birth defects. There is a major concern regarding supplementary vitamin A but not the beta carotene of the dietary form of vitamin A. A relationship was found between high vitamin A consumption during early pregnancy and the occurrence of a variety of birth defects. The data appeared to indicate a teratogenic effect of vitamin A intake not far above the currently recommended dose. Consuming more than 10,000 IU per day was found to be associated with an increased incidence of birth defects when the high levels of vitamin A were taken before the seventh week of gestation (Figure 1). It was estimated that 1.4% of the women in the study averaged more than 10,000 IU of vitamin A per day.

Table 1 and Figure 1 reprinted by permission of *The New England Journal of Medicine;* Rothman K J, et al. *N Engl J Med* 1995;333: 1369-1373.

Editor's comment: *This finding is of great concern because the general public thinks that vitamins are benign and if "a little is good, a lot is better." The study points out there is a fine line between enough and too much. Of particular concern are the additive effects of multivitamins, prenatal vitamins, and fortified foods. Care should be taken by pregnant women or women who wish to become pregnant to limit vitamin A supplementation. In view of the fact that we wish pregnant women to be sure to take sufficient folic acid prior to becoming pregnant and in early pregnancy, the situation can be confusing. It is quite clear that vitamin A can be teratogenic and can be related to other problems besides the classic picture of retinoic acid embryopathy.*

Judith G. Hall, MD

Growth and Physical Outcome of Children Conceived by in Vitro Fertilization

The authors report the status at 2 years of age of 289 Australian children from Victoria who were conceived by in vitro fertilization (IVF). The birth weights of singleton IVF and naturally conceived control infants were similar (IVF: 3,196 g; control: 3,294 g), while the birth weights and gestational ages of IVF twins were slightly greater than those of control twins (IVF: 2,297 g, 35.0 weeks; control: 2,053 g, 33.7 weeks). At 2 years of age, the weight and head circumference percentiles of the entire group of IVF and control children were similar (IVF: 56.3 g; 63.4 cm, respectively; control: 56.2 g; 65.7 cm, respectively). Length percentile of the IVF children was significantly (P=0.004) greater than that of the control children (57.7 cm versus 49.9 cm), the reason for which was not apparent. There was no significant difference between IVF and naturally conceived children with respect to: congenital malformations, subsequent hospitalizations and operations, or neurologic status. The investigators concluded that IVF had no adverse effect on growth, general health, and development at 2 years of age.

Saunders K, et. al. *Pediatrics* 1996;97:688-692.

Editor's comment: *More than 34,000 children have been delivered by assisted reproductive techniques. It is encouraging to note that these interventional methods have produced predominantly normal offspring.*

Allen W. Root, MD

Specification of Pituitary Cell Lineages by the Lim Homeobox Gene *Lhx3*

Lhx3 is a mouse LIM homeobox gene (one associated with morphologic development) that is expressed in the pituitary, hindbrain, spinal cord, and pineal gland. In order to determine the role of this gene in pituitary differentiation, the authors established a model in which this gene has been disrupted and rendered inactive, ie, "knocked out." Animals heterozygous for this mutation are normal and fertile. Although animals homozygous ($Lhx3^{-/-}$) for this recessive mutation are of normal size at birth, they are either stillborn or die within 24 hours, possibly because of abnormalities of respiratory control or adrenocortical function. In $Lhx3^{-/-}$ animals, the pituitary gland fails to form and differentiate properly. Thus, by embryonic day 10.5, the mutant Rathke's pouch differed from the intact, wild-type animal; its opening to the oral cavity was wider and its lining multilayered. As development progressed, Rathke's pouch failed to pinch off from the oral cavity or to develop the histologic appearance of the normal anterior pituitary. The transcription factor *Pit-1* failed to appear in the mutant pituitary anlagen, and thus development of somatotropes, lactotropes, and thyrotropes was disrupted, as was synthesis of growth hormone, prolactin, and β-thyrotropin. In addition, the gonadotropes failed to differentiate in the homozygous ($Lhx3^{-/-}$) mutant animals. Pro-opiomelanocortin (POMC) was detectable in the hypothalamus (the floor of diencephalon) and in a few cells of the mutant pituitary, suggesting that primary corticotrope differentiation was not dependent on *Lhx3*. Inasmuch as proliferation of corticotropes was limited in the mutant animals, this gene may be necessary for further development of this cell line. The authors conclude that *Lhx3* is a critical homeobox gene for anterior pituitary development.

Sheng HZ, et al. *Science* 1996;272:1004-1007.

Editor's comment: *These investigators elegantly describe the consequences of a homozygous mutation in Lhx3 in mice. The human counterpart of this disorder awaits detection, but may be found in patients with pharyngeal pituitaries. Another step in the genetic control of pituitary formation and differentiation has now been identified.*

Lhx3 *maintains expression of Rpx, a homeobox gene necessary for the early stages of pituitary development, and regulates the expression of* Pit-1, *which in turn stimulates the differentiation of a common cellular precursor into thyrotropes and somatomammotropes, the latter further differentiate into somatotropes under the direction of growth hormone-releasing hormone.*

Allan W. Root, MD

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Recombinant Human Growth Hormone Therapy for Children With Chronic Renal Insufficiency: An Update 1996

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Growth retardation as a clinical consequence of uremia, or chronic renal insufficiency (CRI), was identified in the 19th century.[1] Minimal medical attention was devoted to this facet of CRI until the emergence of the dual therapeutic modalities of dialysis and renal transplantation in the 1960s to prolong the lives and effect rehabilitation in a previously uniformly fatal disease process.

Vigorous investigative efforts indicated that the etiology of the growth retardation in children with CRI was multifactorial: (1) age at onset; (2) primary renal disease; (3) fluid and electrolyte abnormalities, especially acidosis; (4) renal osteodystrophy; (5) inadequate caloric intake; and (6) perturbations of growth factors.[2] The last factor has gained increasing significance since the seminal demonstration by Mehls et al [3] in the early 1980s that rhGH improved the growth velocity of growth-retarded uremic rats.

Initial clinical studies in children with CRI (glomerular filtration rate between 5 and 75 mL/min/1.73 m^2) demonstrating the salutary effect of rhGH in improving growth velocity were published in the late 1980s.[4,5] These were followed by short-term (6 months)[6] and long-term (2 years) multicenter, randomized, placebo-controlled studies that validated the safety and efficacy of rhGH in children with CRI. The latter study led the Food and Drug Administration (FDA) to approve, in 1994, the use of rhGH for children with CRI and end-stage renal disease (ESRD) (glomerular filtration rate <5 mL/min/1.73 m^2 and/or the clinical need to initiate dialysis) prior to renal transplantation.

This review will focus on the following pertinent issues regarding the use of rhGH in this patient population: (1) What is the long-term (>5 years) outcome of rhGH use in children with CRI? (2) Is rhGH safe and effective in infants and very young children (<2½ years) with CRI? (3) What is the optimal approach to the clinical management of patients who reach their target height (50th centile for midparental height) while receiving rhGH? (4) Does rhGH accentuate the glucose intolerance associated with CRI? (5) Are there osseous complications associated with the use of rhGH in children with CRI? (6) What factors are responsible for suboptimal responses to rhGH? (7) Should growth-retarded children undergoing dialysis receive rhGH? (8) What is the mechanism for the beneficial effect of rhGH in this patient population?

CME CERTIFICATION:
An Important Change Coming for *GGH*

With the coming of the new year, we have an exciting announcement regarding a change in the status of *GGH*! Beginning with Volume 13, Number 1, *GGH* will be designated by the University of Virginia School of Medicine as a continuing medical education activity certified for Category 1 credit of the Physician's Recognition Award of the American Medical Association. We hope that many of you will find this change will be of value and interest.

In This Issue

LONG-TERM (>5 YEARS) RESULTS

Of the 11 patients included in the initial pilot study, 6 were treated for >5 years and 1 patient is still receiving rhGH after >8 years (Figure 1).[4,5] The magnitude of improvement in growth velocity was not sustained at the same level that was obtained during the initial year of rhGH treatment; however, continued improvement in standardized height was noted during long-term treatment (Figure 2).

Twenty patients (aged 0.7 to 11.3 years) in the multicenter, randomized, placebo-controlled study have been treated with rhGH for >5 years.[7] Growth velocity following initiation of rhGH treatment waned with succeeding years of therapy (Figure 3); however, as with the patients in the pilot study, long-term treatment was associated with a continued beneficial effect on standardized height.

The only significant side effect associated with long-term rhGH treatment was one case of avascular necrosis (AVN). Although there was a decline in the mean renal function during the 5 years of rhGH treatment, it is apparent that there was no acceleration in the magnitude of decline as a consequence of rhGH treatment (Figure 4).

Therefore, the data to date indicate a continued salutary effect of long-term rhGH treatment in children with CRI with minimal adverse events and/or side effects.

Figure 1
Recombinant Human Growth Hormone Treatment in Chronic Renal Failure

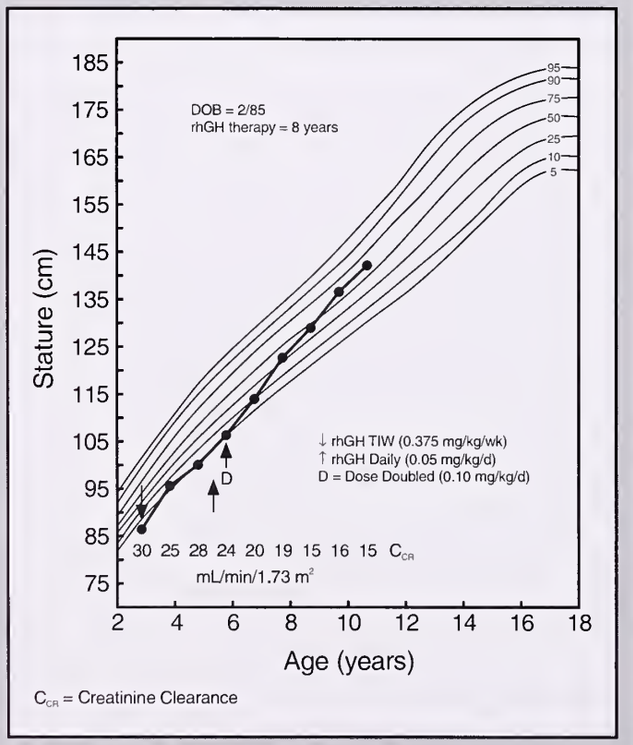
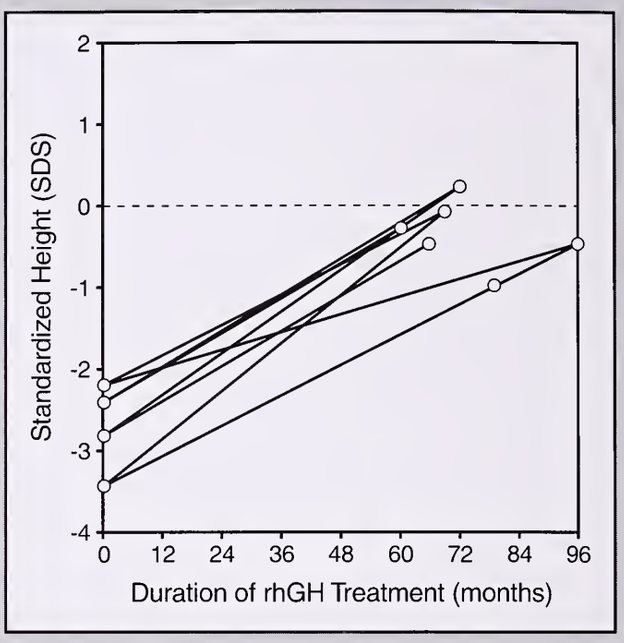


Figure 2
Long-Term (>5 Years) Recombinant Human Growth Hormone Treatment in Chronic Renal Insufficiency: Change in Standardized Height



TREATMENT OF INFANTS (<2 ½ YEARS OF AGE)

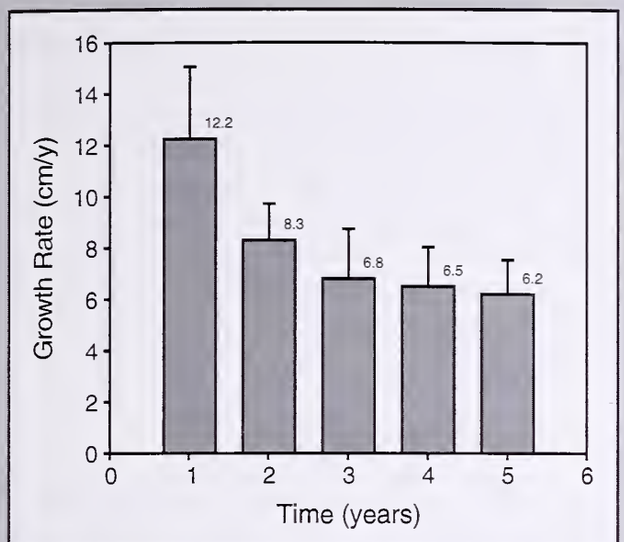
Of the 125 patients with CRI included in the multicenter, randomized, placebo-controlled study,[6] 30 (24%) were <2½ years of age at the initiation of rhGH treatment.[8] The first-year growth rate was 14.1 ± 2.6 cm/y in the rhGH-treated group (n=19) compared with 9.3 ± 1.5 cm/y in the placebo-treated group (n=11, $P<0.00005$). Significant improvement in growth velocity in the rhGH-treated group compared with the placebo-treated group persisted during the second year of the study, albeit to a lesser magnitude ($P<0.025$).

At 2 years, the height SDS was +2.0 ± 0.7 in the rhGH-treated group compared with -0.2 ± 1.1 in the placebo-treated group ($P<0.00005$). No specific adverse events were identified in these young patients receiving rhGH.

These data are consistent with the interpretation that rhGH is safe and effective in young children <2½ years of age with growth retardation secondary to CRI. The youngest patient included in this study was 0.7 years.

It is imperative that potential contributing factors to growth retardation in infants with CRI be corrected *before* the initiation of rhGH in this age group. Fluid and electrolyte imbalances and acidosis should be normalized; optimal nutritional intake should be ensured with the potential reliance upon nasogastric

Figure 3
Recombinant Human Growth Hormone Treatment in Chronic Renal Insufficiency: 5 Year Annual Growth Rate



tube feeding; and renal osteodystrophy should be minimized prior to initiating rhGH treatment.

TREATMENT OPTIONS ONCE TARGET HEIGHT IS REACHED

Once the target height (50th percentile for midparental height) is reached, there are potentially 3 options available to sustain optimal standardized height (target height): (1) continue administration of rhGH at the current dosage schedule; (2) discontinue rhGH and observe for fluctuation in standardized height, reinstituting rhGH if the standardized height falls below an arbitrary level (<25th centile for midparental height); and (3) continue administration of rhGH at an arbitrary lower dosage.

Data from the multicenter study[9] would tend to support the second option. Twenty-two children were "paused," ie, rhGH was temporarily discontinued, because the patients' target height was reached. Six of the 22 children remained paused at the time of the report for a mean ± SD duration of 25.5 ± 26.9 months; however, 16 of the 22 children required resumption of rhGH after pausing for a mean ± SD of 9.0 ± 4.6 months because of a reduction in standardized height. During the pause, the latter group of patients grew only a mean ± SD of 2.7 ± 1.7 cm/y, following resumption of rhGH, the growth velocity increased to 7.2 ± 1.7 cm/y. Although 73% (16 of 22) of children who were paused following attainment of target height required reinstitution of rhGH, it appears that discontinuation of rhGH once target height is reached is the optimal strategy, since 27% of the children had sufficient subsequent growth velocity to sustain their target height without additional rhGH therapy.

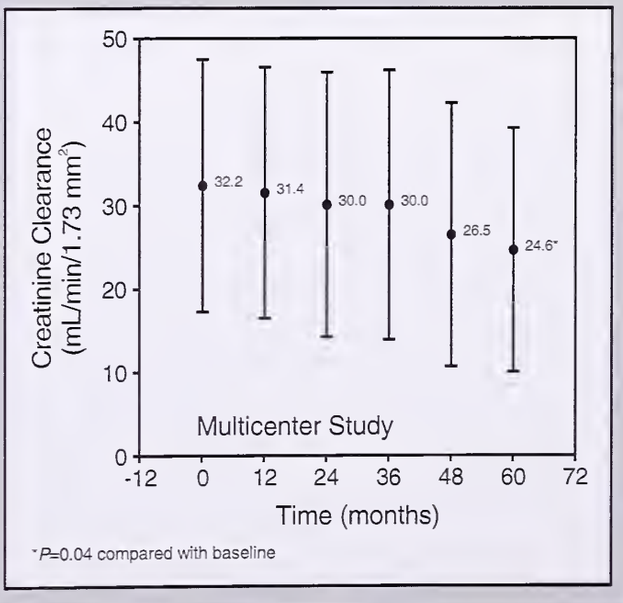
GLUCOSE INTOLERANCE

In the long-term (>5 years) follow-up of 20 growth-retarded children with CRI treated with rhGH in the multicenter study,[7] there was no significant change in the mean fasting glucose or 2 hour postprandial glucose values compared with baseline at any time interval following initiation of rhGH treatment.

The fasting plasma insulin levels were significantly increased compared with baseline levels at each interval following initiation of rhGH treatment. Similarly, the 2 hour postprandial plasma insulin levels were significantly increased compared with baseline levels at 24, 48, and 60 months of rhGH treatment. The mean ± SD HbA_{1c} level was 5.3 ± 0.9% at baseline and 6.1 ± 0.9% at 5 years (P=0.0003). To date, there have been no clinical consequences associated with the hyperinsulinemia in patients with CRI treated with rhGH.

Saenger et al[10] evaluated carbohydrate metabolism by fasting and postprandial glucose, insulin, and HbA_{1c} levels in patients with either CRI, GHD, Turner syndrome, or ISS who were treated with rhGH for 5 years. Some of the children with CRI were probably included in the long-term multicenter study report. Mean fasting and postprandial glucose values remained unchanged throughout the 5-year term in all 4 groups. Mean fasting and postprandial

Figure 4
Recombinant Human Growth Hormone Treatment in Chronic Renal Insufficiency: 5 Year Annual Creatinine Clearance Data



insulin values rose yet remained within the normal range at 5 years. The mean HbA_{1c} levels in the CRI patients were slightly elevated to 6.3% at 5 years. This comparative study indicated that carbohydrate metabolism was not adversely impacted by rhGH in CRI patients compared with other groups of patients treated with rhGH.

OSSEOUS COMPLICATIONS

Data from the multicenter study indicated no significant difference in radiographic osteodystrophy scores or in serum calcium, phosphorus, or parathyroid hormone (PTH) levels between the rhGH and control groups.[11] However, the increment in growth velocity in response to rhGH treatment was blunted in the presence of secondary hyperparathyroidism.[12]

Slipped capital femoral epiphyses (SCFE) were noted in 2 of the 125 patients included in the multicenter study; 1 child was treated with rhGH for 3 years and 1 for only 3 months at the time of diagnosis. Both patients had severe renal osteodystrophy.

Watkins et al[13] noted AVN in 6 of 17 children with CRI receiving rhGH; 3 had AVN prior to rhGH treatment and 3 had no previous radiographs. Similarly, Mehls et al[14] reported 2 cases of AVN in 103 prepubertal patients with CRI treated with rhGH. Boechat et al[15] reviewed the radiographs of 205 children included in multicenter studies of rhGH therapy in CRI and detected 15 cases of AVN; 8 had AVN prior to rhGH and 7 had no prior radiographs.

Consequently, the risk of SCFE and/or AVN in children with CRI receiving rhGH remains equivocal. Nonetheless, it is prudent to obtain radiographs of the osseous structures prior to initiating rhGH in children with CRI and to repeat the radiologic studies if clinical symptoms ensue.

Similarly, it is advisable to correct the radiologic and/or serologic abnormalities of renal osteodystrophy prior to initiating rhGH treatment. Persistent renal osteodystrophy may blunt the response to rhGH.

SUBOPTIMAL RESPONSE TO rhGH

There are few data detailing the incidence of suboptimal responses to rhGH in children with CRI during the initial year of treatment. Almost without exception all previous reports have noted a decline in the growth velocity during the second and all succeeding years of rhGH treatment. Despite this decline, continued improvement in standardized height (Figure 2, page 50) has occurred.

Potential causes of suboptimal responses either initially or subsequently include the following: (1) failure to correct other contributory causes of growth retardation in children with CRI, ie acidosis, fluid and electrolyte abnormalities, and inadequate caloric intake; (2) persistent renal osteodystrophy, as evidenced by an inverse correlation between PTH level and growth velocity; (3) level of renal functional impairment, ie, ESRD patients may have a decreased response compared with CRI patients; and (4) noncompliance. Serial measurements of IGF-1 may be helpful in detecting the latter. Failure to demonstrate an increase in the IGF-1 level in response to presumed rhGH administration may indicate noncompliance.

In a few instances, upward adjustment of the rhGH dosage has proven effective in improving growth velocity.[16] However, prior to contemplating adjustments in dosage, it is imperative that the other potential causes of suboptimal response be investigated and corrected.

rhGH IN DIALYSIS PATIENTS

There are limited data detailing the use of rhGH in growth-retarded children undergoing dialysis. Unfortunately, no controlled studies have been undertaken; however, Phase I studies have uniformly demonstrated a significant improvement in growth velocity during the initial year of rhGH treatment compared with that obtained during the year prior to treatment.[14,17,18] These studies primarily included patients undergoing peritoneal dialysis.

During the second year of rhGH treatment, growth velocity diminished substantially to a level comparable to that seen during the year prior to treatment.[14,18] Reasons for this marked reduction in response were not apparent; however, it has been suggested that progressive renal osteodystrophy, persistent acidosis, reductions in residual renal function, and noncompliance may all be contributory.

Limited data are available utilizing rhGH in children undergoing hemodialysis.[19] Comparison of

In Future Issues

Status of and Indications for Leg Lengthening Procedures
Deborah Stanitski, MD

The Neuroendocrinology of Stress in Its Relation to Alterations in Growth
George Chrousos, MD

The Pathophysiology of Growth Failure in Renal Disease
David Powell, MD

Update Article Pertaining to the Genes of Growth Factors and Hormones
Victor McKusick, MD

results obtained in children undergoing hemodialysis, continuous ambulatory peritoneal dialysis (CAPD), or automated peritoneal dialysis (APD) seem to indicate similar efficacy.

At least one report has indicated that the response to rhGH in children undergoing dialysis is less than that achieved in children with CRI.[20] However, others have not substantiated this finding.[6]

The lack of uniform, long-term, continued improvement of growth velocity and/or standardized height in children undergoing dialysis who are receiving rhGH has led to questioning of the justification for rhGH treatment in the pediatric dialysis population. This is an important issue since approximately 50% of the pediatric patients undergoing dialysis in North America have a SDS exceeding -1.88.[21]

MECHANISM OF rhGH EFFICACY IN CRI

The current hypothesis proposes that rhGH increases the level of bioavailable (free) IGF-1, which subsequently stimulates bone growth. Endogenous growth hormone (GH) levels are elevated in CRI primarily as a result of reduced renal clearance, since GH secretory rates are normal.[22] Despite elevated GH levels, IGF-1 secretory rate by the liver is reduced, possibly as a result of a reduction in the number of hepatic GH receptors. This is reflected by a reduction in GH-binding protein levels in uremia. Furthermore, IGFBP levels, primarily IGFBP-2 and possibly IGFBP-3, are elevated, which reduces the levels of available free IGF. rhGH increases the IGF levels to a greater extent than it increases the IGFBP levels, thereby effectively increasing levels of free IGF to enhance bone growth.

SUMMARY

1. Long-term (>5 years) rhGH treatment in children with CRI produces sustained improvement in standardized height.
2. rhGH treatment of infants (<2½ years of age) with CRI is as effective at improving growth velocity as it is in older children with CRI.
3. Once target height (50th centile for midparental height) is reached, the optimal approach is to pause rhGH treatment and observe the patient. If standardized height declines significantly, ie, -2 SDs, rhGH is effective when reinitiated.
4. Neither short-term nor long-term rhGH treatment in children with CRI adversely impacts on glucose tolerance.
5. The presence of renal osteodystrophy may blunt the impact of rhGH and increase the risk of SCFE and/or AVN in children with CRI. Pretreatment radiologic evaluation and radiologic surveillance of clinical symptoms is indicated.
6. Suboptimal response to rhGH in children with CRI is rare and may indicate the need for upward dosage adjustment. However, prior to this, potential causes of growth failure in CRI should be corrected.
7. rhGH is effective during the initial year of treatment. Growth velocity may be blunted during subsequent years of treatment. The precise mechanism of this blunting has not been delineated.
8. rhGH has been shown to improve growth velocity in patients undergoing either peritoneal dialysis or hemodialysis; however, long-term data are lacking and the response may be less than that achieved in patients with CRI.
9. rhGH is probably effective in infants, children, and adolescents with CRI because it increases the levels of bioavailable (free) IGF.

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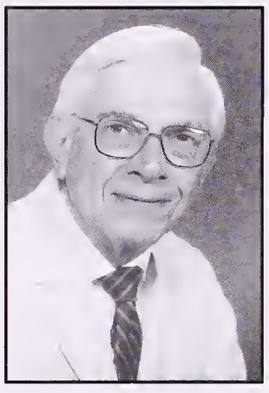

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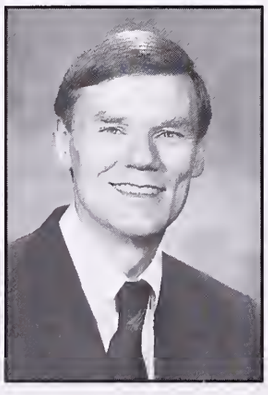

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Abstracts From the Literature

Nonsense Mutation in the Human Growth Hormone-Releasing Hormone Receptor Causes Growth Failure Analogous to the Little (*lit*) Mouse

Growth hormone-releasing hormone (GHRH) was identified in 1982 as a potent stimulus for GH-secretion. The synthesis and secretion of GH in the anterior pituitary is regulated by the hypothalamus through GHRH and somatostatin. GHRH stimulates the secretion of GH while somatostatin inhibits its secretion. These hormones bind to the GHRH receptors (GHRHRs) and control the synthesis and secretion of GH.

Wajnrajch and coworkers have identified a nonsense mutation within the GHRHR in a family with proportionate short stature. Although the clinical and laboratory features resulting from this mutation mimic those of GH-deficient individuals, these patients are capable of making GH but not releasing it. Their short stature is due to a nonfunctioning GHRHR caused by the mutation. This mutation is responsible for unresponsiveness to exogenous GHRH and consequent growth failure. The *GHRHR* gene was identified in 1992 and was mapped to chromosome 6 in mice. Human *GHRHR* shows strong sequence homology to the murine gene. In humans, the gene is mapped to chromosome 7p15.

The authors reported nonsense mutations in the *GHRHR* gene in 2 members of a consanguineous family. Mutations of *GHRHR* gene must be considered as a cause for clinical features suggestive of GH deficiency in cases in which the GH gene (*GH1*) itself is normal. The mutation is a Glu72Stop mutation and its position is close to the *lit* mutation (Asp60Gly) identified in mice in 1992 and mapped to chromosome 6 in mice. The mutation occurs in the same highly conserved region of the extracellular domain.

In this report, Wajnrajch et al have identified a nonsense mutation in the GHRHR in humans for the first time. The Glu72Stop mutation produces a severely truncated GHRHR that lacks the G-protein sites. This produces a disruption of hormonal signals. Patients with this mutation respond to exogenous GH but not to GHRH. The identification of this mutation causing GHRH dysfunction suggests that both the GH-releasing peptide (not GHRH) and nonpeptididyl benzazapines might be useful therapeutic agents in these disorders because both stimulate GH release independent of the GHRHR.

The GHRHR may play a role in prolactin synthesis in the mouse, as evidenced by reduced levels in the *lit* mouse. However, the baseline and thyroid-releasing hormone-stimulated prolactin levels were tested and were normal in this family.

Receptor-activating mutations also should be looked for in GH excess diseases such as acromegaly. Activating mutations in the stimulatory G-protein α subunit, to which the GHRHR is functionally coupled, is seen in some acromegalic patients. Activating mutations in related G-protein coupled receptors occur in human diseases, including one in the LH-R in one type of male precocious puberty (testotoxicosis), one in the TSH-R in congenital persistent thyrotoxicosis, one in the PTH-R in metaphyseal chondrodysplasia, and one in the calcium-sensing receptor in dominant hypocalcemia.

Wajnrajch MP et al. *Nature Genet* 1996;12:88-90.
Mayo KE. *Nature Genet* 1996;12:8-9.

***Editor's comment**: The discovery of mutations such as reported here, which help to define metabolic pathways, are very satisfying. In the future, additional mutations will enhance our knowledge regarding the diagnosis and treatment of syndromes with hormonal deficiency and excess.*

Growth problems and short stature are a common pediatric problem. Mutation of intermediate processing steps such as GHRH binding do exist. It is as yet unclear how common this problem is, but it must be considered in all apparent GH-deficient children who do not respond to GHRH with GH release.

Judith G. Hall, MD

***2nd Editor's comment:** A second report has already been made.[1] A cluster of severe dwarfism has been described in Pakistan. A total of 18 dwarfs was discovered in a kindred with high consanguinity. Inheritance is autosomal recessive, and the dwarfism severe (114 to 136 cm). Biochemical and endocrine evaluation was consistent with isolated GH deficiency (no GH response to GHRH, clonidine, L-dopa, or TRH). IGF-1 was extremely low (<10 ng/mL), as was IGFBP-3. Both responded well to GH. The* GHRHR *locus on chromosome 7p15 was highly linked to the dwarfed phenotype. It appears that this form of dwarfism is caused by an inactivating mutation in the* GHRHR *gene, and that this entity represents a human homologue of the little* (lit/lit) *mouse.*

Robert M. Blizzard, MD

1. Maheshwari H, et al. *The Endocrine Society Program Book.* 1996;Abstract OR46-2:709.

Functional Activation of Mutant Human Insulin Receptor by Monoclonal Antibody

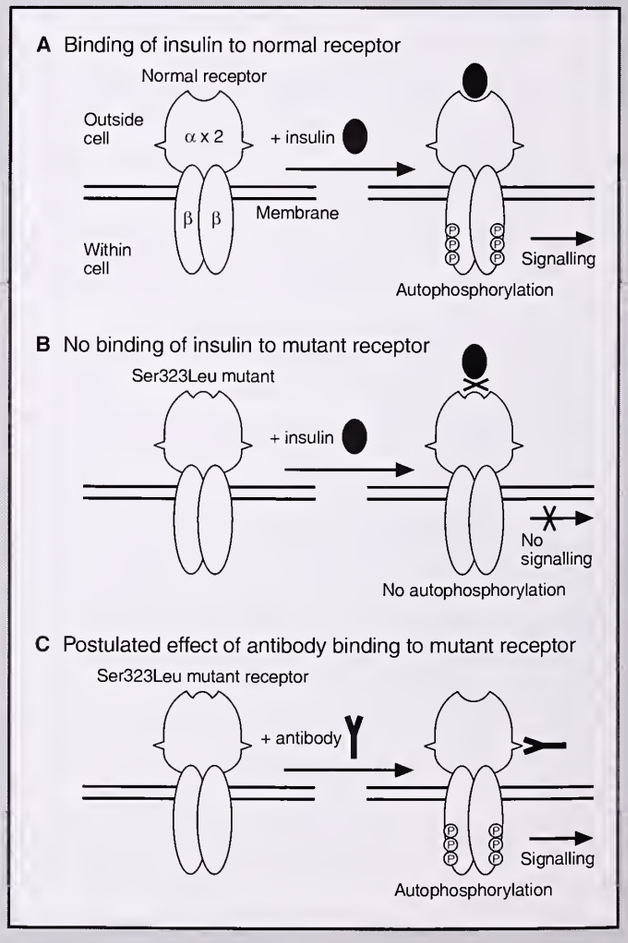
The investigators have identified a mutation (Ser323Leu) in the extracellular, ligand-binding domain of the insulin receptor that resulted in decreased binding of insulin and consequently severe insulin resistance (Rabson-Mendenhall syndrome). Although biologically inert, this mutant receptor is normally inserted into the insulin target cell membrane. The authors generated a monoclonal antibody to sequence 485-592 of the extracellular domain of the insulin receptor. They demonstrated that this antibody bound to and induced autophosphorylation not only in wild-type insulin receptor but also in the mutant insulin receptor expressed in Chinese hamster ovary cells. Cells transfected with the wild-type and mutant insulin receptors were also able to synthesize glycogen in response to this antibody. The authors suggest that it may be possible to treat patients with this form of insulin receptor defect with a stimulatory monoclonal insulin receptor antibody or to design drugs that bypass the defective ligand binding site (Figure 1).

Krook A, et al. *Lancet* 1996;347:1586-1590.

***Editor's comment:** Many genetic defects in cell membrane receptors lead to impaired synthesis, extreme shortening or abnormal folding of the translated protein, and hence failure of its insertion into the cell membrane. However, in those hormone resistance syndromes in which the receptor defect involves the extracellular domain and permits its translocation into the cell membrane, generation of receptor-stimulating antibodies may present a significant therapeutic option. In patients with insulin-resistant diabetes mellitus, IGF-1 has been utilized with success. However, concerns remain about the long-term consequences of the administration of this potent growth factor.*

Allen W. Root, MD

Figure 1



Postulated mechanism by which monoclonal antibody to receptor could substitute for ligand in the case of a mutant insulin receptor that does bind insulin.

Putting the Brakes on Bone Growth

A fascinating story is emerging regarding the local control of linear bone growth. It has long been recognized that chondrocytes in skeletal growth plates progress through a complex differentiation process that involves proliferation and terminal differentiation (hypertrophy). Moreover, although a number of hormones and growth factors, most notably GH and IGF-1, are known to influence this progression, the local controls have remained poorly understood. Now, papers from 3 Boston research teams have defined a local negative feedback loop that serves as a brake on this process, controlling the rate of terminal chondrocyte differentiation (see Figure 1).

The feedback loop is simple; the proof of its existence was much more difficult. The loop has 2 major players: a signaling molecule called Indian hedgehog (Ihh) and parathyroid hormone-related protein (PTHrP). Ihh is 1 of at least 3 hedgehog proteins found in higher vertebrates that function as signal proteins, especially during early embryologic development. Hedgehog signals are thought to act through a receptor known as Patched (Ptc) and a transcription factor named Gli.

Through an elaborate series of experiments in developing chick limb buds and mouse embryos in which relevant genes were overexpressed and/or inactivated, the authors were able to determine the upstream and downstream relationships of loop components. First, they demonstrated that Ihh was produced by growth plate chondrocytes when they begin to terminally differentiate and that overexpression of Ihh suppressed terminal differentiation. Next, they showed that Ptc receptor and Gli transcription factor were expressed in perichondrial cells around the periphery of the growth plate.

Related experiments showed that PTHrP is synthesized by periarticular perichondrial cells and that PTHrP receptor, which is also a receptor for PTH, is expressed by proliferating chondrocytes just prior to terminal differentiation. Genetic inactivation of the receptor was associated with accelerated chondrocyte terminal differentiation. Finally, the loop was closed when Ihh suppression of terminal differentiation was shown to depend on PTHrP.

The proposed model is shown in Figure 1. Briefly, as growth plate chondrocytes decide to terminally differentiate, they express high levels of PTHrP receptor. Once committed to this fate, they transiently express Ihh, which acts on the adjacent perichondrial cells through Ptc and Gli to directly or indirectly cause periarticular perichondrial cells to secrete PTHrP. PTHrP signals back to proliferating chondrocytes expressing PTHrP receptors, preventing them from progressing down the terminal differentiation pathway. Thus, the loop functions as a brake on terminal differentiation, essentially controlling the number of cells terminally differentiating at any given time.

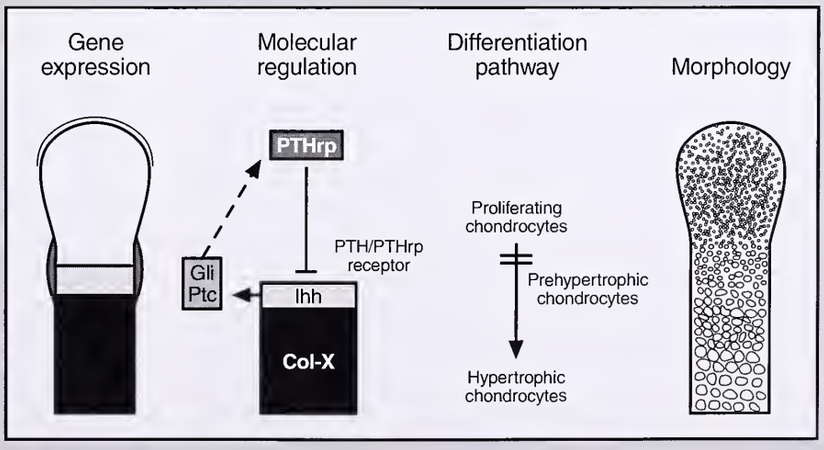
Roush W. *Science;* 1996;273:579.
Vortkamp A, et al. *Science* 1996;273:613-622.
Lanske B, et al. *Science* 1996;273:663-666.

Editor's comment: *This work provides a new context in which to consider control of bone growth. The greatest uncertainty is how Ihh signals to periarticular perichondrial cells that secrete PTHrP. Nevertheless, that PTHrP is required for Ihh inhibition of chondrocyte terminal differentiation is hard to dispute. Given the long distances in this model that PTHrP must diffuse through cartilage matrix, a recognized barrier to diffusion of many molecules—especially in larger bones such as those in humans—it is difficult to imagine how this feedback loop would be responsible for the fine-tuning of subtle events in the growth plate. As the authors imply, perhaps this loop is one of several locally acting mechanisms that control skeletal development and growth.*

William A. Horton, MD

Figure 1
Proposed Regulation of Cartilage Differentiation During Bone Growth

Ordinarily, growth plate chondrocytes become hypertrophic chondrocytes, briefly passing through a prehypertrophic stage during which they sequentially express the PTH/PTHrP receptor and Ihh genes. The Ihh signal is transmitted to the perichondrium, where it elicits expression of another set of genes, Gli and Ptc, which leads to expression of PTHrP in the periarticular perichondrium. PTHrP then signals back to its receptor in the prehypertrophic cells to block progression of more cells down the hypertrophic chondrocyte pathway, ie, it closes the negative feedback loop. As chondrocytes fully hypertrophy, Ihh expression ceases, which releases the "brake" imposed by the negative loop, allowing more cells to enter the hypertrophic pathway.



Reprinted with permission from Vortkamp A, et al. Regulation of Rate of Cartilage Differentiation by Indian Hedgehog and PTH-Related Protein. *Science* 1996;273: 613-666.

Skeletal Overgrowth and Deafness in Mice Lacking Fibroblast Growth Factor Receptor 3

Molecular defects in fibroblast growth factor 3 receptor (FGFR3) have been found in patients with achondroplasia, hypochondroplasia, thanatophoric dwarfism, and Crouzon syndrome—dysplasias that adversely affect formation of endochondral bones (long bones, base of the skull, vertebrae). In mouse embryos, the gene for FGFR3 (*Fgfr3*) is expressed not only in cartilage but also in glial cells of the brain and spinal cord and in the cochlea. Colvin et al developed mice homozygous for absence of expressed FGFR3 by engineering a truncated *Fgfr3* that lacked the coding regions for its extracellular and transmembrane domains. Although *Fgfr3*$^{-/-}$ mice survived gestation and birth, 48% died within 21 days after delivery; however, some lived as long as 8 months. Kinking of the tail, kyphosis, scoliosis, increased femoral and humeral length and curvature, and abnormal rib formation developed in >75% to 100% of *Fgfr3*$^{-/-}$ mice. Histologic examination of the cartilage growth plate of the long bones revealed enlargement (+33% to 50%) of the hypertrophic zone in *Fgfr3*$^{-/-}$ mice. The authors attributed the skeletal abnormalities in *Fgfr3*$^{-/-}$ mice to disordered cartilage cell growth, development, turnover, and replacement by endochondral ossification and concluded that FGFR3 regulates these processes. Because the morphologic and histologic findings in *Fgfr3*$^{-/-}$ mice are the converse of those seen in patients with achondroplasia, the investigators suggest that this disorder is the result of constitutive activation of FGFR3 due to the mutation (Gly380Arg) in its transmembrane domain. In addition to the skeletal deformities noted above, abnormalities of cochlear formation and hearing were present in *Fgfr3*$^{-/-}$ mice. In these animals, the organ of Corti failed to differentiate and progress from the neonatal state. Thus, FGFR3 is also necessary for normal development of the organ of Corti and hearing.

Colvin JS, et al. *Nature Genet* 1996;12:390-397.

Editor's comment: *The data presented in this elegant paper indicate that FGFR3 affects cartilage formation, maturation, and endochondral bone formation by regulating the size of the hypertrophic zone of growth plate cartilage, its invasion by blood vessels preparatory to ossification, and the turnover of cartilage cells. In achondroplasia, proximal long bones of the extremities (humerus, femur) are shortened, and the height of the hypertrophic zone of the cartilage growth plate is decreased. These findings are opposite to those present in* Fgfr3$^{-/-}$ *mice. The authors' suggestion that achondroplasia is the result of constitutive activation of FGFR3 is supported by data reported by Webster and Donoghue*[1] *and Naski et al.*[2] *These investigators transfected cells in cultures with FGFR3 with the mutations present in patients with achondroplasia (Gly380Arg, in the transmembrane domain) and in subjects with thanatophoric dysplasia (Arg248Cys, in the extracellular domain, and Lys650Glu, in the second tyrosine kinase region of the intracellular domain). In the absence of ligand (FGF1), there was proliferation of the transfected cells and dimerization and autophosphorylation of the FGFR3, indicative of the constitutive activation of the mutated FGFR3. (Interestingly, the Gly380Arg mutation leads to cellular proliferation in transfected cells, but apparently decreased proliferation of chondrocytes in vivo. This discrepancy requires explanation.) One mutation of FGFR2 present in some patients with Crouzon syndrome results in its constitutive activation as well.*[3]

Allen W. Root, MD

1. Webster MK, Donoghue DJ. *EMBO* 1996;15(3):520-527.
2. Naski MC, et al. *Nature Genet* 1996;13:233-237.
3. Neilson KM, Friesel RE. *J Biol Chem* 1995; 270:26037-26040.

Molecular Definition of Breakpoints Associated With Human Xq Isochromosomes: Implications for Mechanisms of Formation

An isochromosome is a type of chromosomal aberration in which one of the arms is duplicated and the other arm is deleted; both the arms have the same set of genes but in a reverse sequence. Isochromosome for Xq is the most common structural abnormality observed in Turner syndrome, which results in a duplication of the long arms of X. About 15% of Turner syndrome patients have an i(Xq) in mosaic or nonmosaic form.

Wolff et al have brought to light a new mechanism for isochromosome formation. They studied 11 i(Xq)s derived from Turner syndrome patients using molecular techniques and found that the isochromosomes are not usually due to misdivision of the centromere as previously thought (Figure 1). Instead, they are formed after Xp breakage and a U-type reunion event in the pericentromeric region. Using fluorescent in site hybridization (FISH) techniques, they have localized the breakpoints in the band Xp11.2. The data support the hypothesis that structurally dicentric i(Xq)s initially contain 2 functional centromeres, resulting in the loss of the i(Xq) in some cells during the early divisions of the zygote. According to this hypothesis, those cells that maintain the i(Xq) chromosome inactivate 1 of the centromeres, conferring stability.

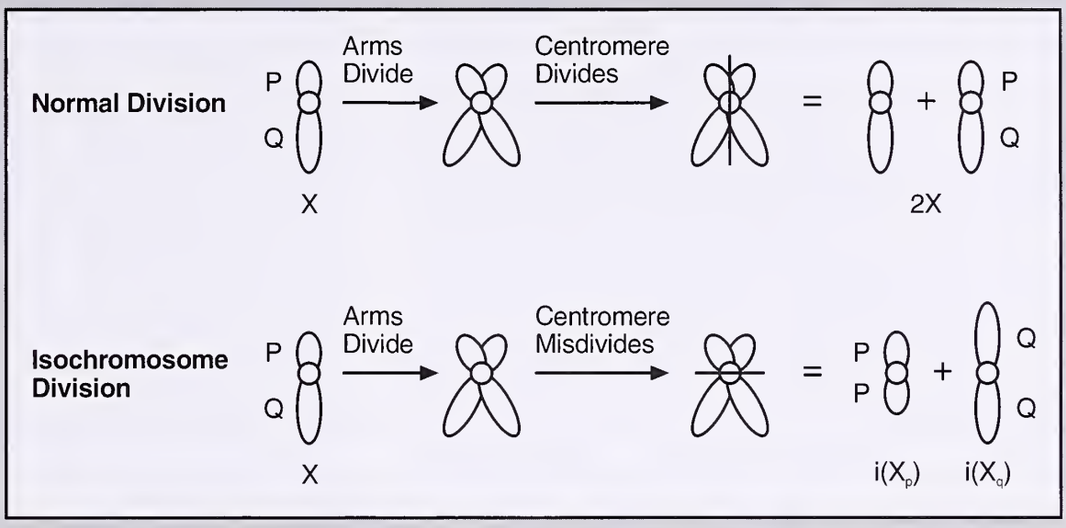
Wolff DJ, et al. *Am J Hum Genet* 1996;58:154-160.

Editor's comment*: This is a breakthrough in our understanding of the mechanisms of isochromosome formation and supports some previous studies. More studies are needed to find out whether other regions of breakpoints on the X chromosome and other mechanisms for isochromosome formation occur. Investigation defining whether the breakage follows*

a particular nucleotide sequence, or is sequence dependent, also will help clarify X chromosomes that are predisposed to isochromosome formation. It also may help to determine whether certain X chromosomes are more predisposed to producing germ cells or zygotes with sex chromosome loss, addition, or changes. The hypothesis cited above regarding inactivation of 1 centromere in a dicentric i(Xq) requires further study.

Judith G. Hall, MD

Figure 1
Previous Concept



This concept may be outdated if report by Wolff et al is confirmed for all instances of i(X_q).

A Regression Method Including Chronological and Bone Age for Predicting Final Height in Turner's Syndrome (PTS), With a Comparison of Existing Methods

Van Teunenbroek et al present a new method for predicting final height (FH) in girls with Turner syndrome using either the Greulich and Pyle (GP) or Tanner and Whitehouse (TW) bone age determinations. The predicted final height in these Turner girls was either PTS by Gruelich Pyle (PTS_{GP}) or by TW using radius, ulna, and short bones (PTS_{RUS}). To develop their regression equations, they utilized data from 57 Dutch women (235 measurements points). These women were born between 1934 and 1973 and, with the exception of estrogen, received no other growth-promoting agents. Criteria for the achievement of final height included: (1) a follow-up to at least age 20 years; or (2) a height velocity of <0.5 cm over the previous year; or (3) a height velocity of <1 cm over the previous 2 years and a bone age (TW) of at least 15 years of age. The PTS, which they developed, can be calculated as follows: FH (final height in centimeters) = a × H (actual height) + b × CA (chronologic age) + c × BA (bone age) plus a constant. Smoothed regression coefficients and constants were created for chronologic ages 6 through 19 years for both the TW and GP systems. A prediction error was calculated to compare other prediction methods with this new equation. The mean prediction errors of both the PTS_{RUS} and the PTS_{GP} were small and similar except for the chronologic ages of 15 through 18 years. There was an overall tendency to over predict final height; however, the mean error of all final height predictions was less than for the Bailey-Pinneau (BP) methods.

Van Teunenbroek A, et al. *Acta Pediatr* 1996;85:413-420.

***Editor's comment**: The authors point out the importance of having a single variable prediction method for FH in girls with Turner syndrome. In addition, they restate that BP and TW methods were developed from data on healthy children and included predictions of a pubertal growth spurt. Thus, these methods are not particularly useful in the prediction of FH in girls with Turner syndrome. Accurate FH predictions could be useful in deciding whether to initiate growth hormone therapy and in evaluating the effects of growth hormone and other anabolic agents on FH.*

I agree with the authors' conclusions: "Of the single-variate FH prediction methods, the smallest mean prediction errors at most ages were observed using the modified PAH [projected height], with a good accuracy from the age of 9 years onwards. Averaging mPAH [modified PAH] with methods allowing for BA increased the accuracy of the more inaccurate method substantially. Thus, if population-specific Turner reference data are available, a number of calculations (with possible errors) can result in a smaller mean prediction error and a higher accuracy. On the other hand, the simplest methods—the mPAH and PAH—were remarkably good at most ages." This article should be read by all groups evaluating the effects of therapeutic agents on the ultimate heights of children.

William L. Clarke, MD

The Role of Proteoglycans in Overgrowth Syndrome

Editor's comment: *The comment is presented before the abstract to alert the reader to the importance of the topic.*

Beckwith-Wiedeman syndrome (BWS) is characterized by intrauterine overgrowth but normal adult stature, hemihyperplasia, and an increased incidence of a variety of embryonal tumors; Simpson-Golabi-Behmel syndrome (SGBS) is associated with prenatal and postnatal overgrowth, resulting in tall adult stature (often males reach >195 cm); cleft lip/palate; polydactyly; vertebral, rib, and sternal malformations; congenital heart disease; cryptorchidism; and hypospadias. The 2 syndromes overlap as both display macroglossia, omphalocele, and an increased incidence of Wilms' tumor. BWS is associated with overexpression of paternally imprinted IGF-2 (paternal heterodisomy or isodisomy, maternal deletion of 11p15.5).

Glypicans are proteoglycans containing complex sugar molecules, such as dermatan, chondroitin, and heparin sulfate, that are anchored to the exterior of the cell membrane through glycosylphosphatidylinositol links. Four molecules currently comprise the human glypican-related integral membrane proteoglycans (GRIPS) family. Glypican-3 may modulate the growth-promoting effects of IGF-2 by acting as a coreceptor with the IGF-2 (mannose-6-phosphate) receptor (Figure 1). In the absence of glypican-3, the growth-promoting effects of IGF-2 may be unregulated. It will be interesting to learn if abnormalities in GPC3 *are found in other overgrowth syndromes such as cerebral gigantism.*

Allen W. Root, MD

The investigators have identified deletions in the gene *GPC3* for a cell-surface proteoglycan termed glypican-3 in patients with SGBS. SGBS is an X-linked (Xq26) recessive overgrowth syndrome related to, but distinct from, BWS (see above). Glypican-3 is a 580 amino acid protein whose gene contains 8 or more exons. Studying female patients with SGBS and translocations between the long arm of the X chromosome and autosome 1 and 16, the authors identified the *GPC3* gene and its deletions at the translocation breakpoint. Gene sequence was 94% homologous with a previously identified rat cell-surface proteoglycan, thus permitting characterization of the *GPC3* product as a glypican. Utilizing *GPC3* probes, the investigators detected deletions of 1 to 3 exons in 3 families with male-limited SGBS. They did not detect gross exon deletions in 3 other families, suggesting that in their affected members more subtle mutations (point mutations) in *GPC3* may be present (or that another gene defect exists with a similar phenotype to that of SGBS). *GPC3* is expressed primarily during embryologic development in mesenchymal tissues (lung, kidney, liver) and not in brain or white blood cells. With an antiserum against glypican-3, the authors demonstrated that this proteoglycan associated with IGF-2 and its binding protein(s). They suggest that SGBS may be due to defective binding of IGF-2 by glypican, thus permitting IGF-2 to exert unrestrained growth-promoting effects during embryologic development and tumor formation in later life.

Pilia G, et al. *Nature Genet* 1996;12:241-247.
Weksberg R, Squire JA, Templeton DM. *Nature Genet* 1996;12: 225-227.

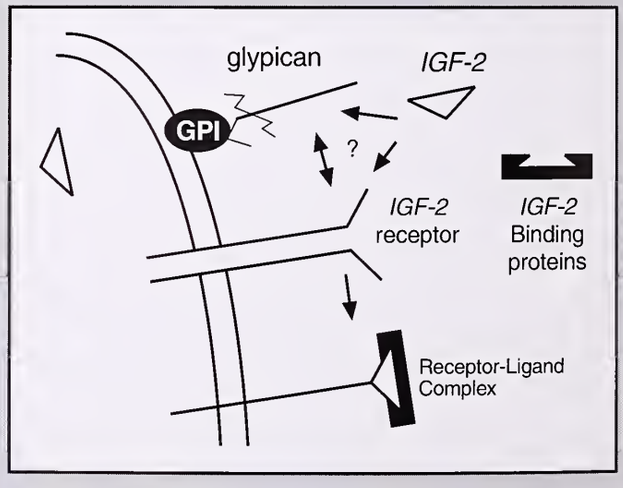
Second Editor's comment: *Several points are made in this article. First, diagnoses of rare syndromes are not always what they seem, or are said to be, even when registered in the NIGMS repository. Investigators who use cell lines from this repository should keep this in mind when studying cells from patients whose diagnoses are difficult to make and for which specific criteria evolve over time, as commonly occurs for rare dysmorphic syndromes.*

Secondly, we are reminded to keep an open mind about molecules and their biologic functions. For example, proteoglycans were originally considered boring molecules that primarily occupied space in connective tissues. Now it appears that some proteoglycans may play important roles in ligand-receptor interactions of growth factors. Another example of this phenomenon involves another proteoglycan, heparin sulfate, which appears to be required for FGF ligands to bind their receptors.

Finally, the report demonstrates how several different disciplines can interact to advance the understanding of a process that may be an important regulator of growth. Indeed, this work could not have been completed without collaborations among dysmorphologists, endocrinologists, gene mappers, and molecular biologists.

William A. Horton, MD

Figure 1



Putative role of glypican-3 in the modulation of IGF-2 interactions with IGF-2R. The figure depicts IGF-2 interactions at the cell membrane with homodimeric IGF-2R and the potential role of glypican-3 in facilitating ligand association. IGF-2 binding proteins are depicted as rectangles that can chaperone IGF-2 (triangles) to dimerized IGF-2R. Glypican-3 is anchored to the peripheral membrane by the GPI linkage shown as a black circle.

Mutations in the Ca^{2+}- Sensing Receptor Gene Cause Autosomal Dominant and Sporadic Hypoparathryoidism

The 7-transmembrane, G-protein-associated, Ca^{2+}-sensing receptor gene is present on chromosome 3q. Abnormalities in this gene have been associated with familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia of varying severity. Baron et al have identified 3 mutations in this receptor: Gln681His (first extracellular loop) and Ala116Thr (amino terminal, extracellular domain) in 2 different families with autosomal dominant hypocalcemia and Phe806Ser (sixth transmembrane domain) in a third patient but with sporadic hypocalcemia. Symptoms varied from muscle cramping to neonatal seizures. All had hypercalciuria despite hypocalcemia, reflecting the role of the Ca^{2+}-sensing receptor in the modulation of renal calcium excretion. The authors point out that conventional treatment of this disorder with calcitriol with or without supplemental calcium may increase urinary calcium excretion. Thus, for optimal treatment of this disorder it may also be necessary to administer an agent that lowers urine calcium excretion (a thiazide).

Baron J, et al. *Hum Mol Genet* 1996;5:601-606.

Editor's comment: *The reader is referred to Dr. Shenker's article,* Activating Mutations in G Protein-Coupled Signaling Pathways As a Cause of Endocrine Disease (GGH *1996;12[3]: 33-38). These subjects are closely related. The reader also may wish to read a comprehensive review by Pearce and Brown concerning defects of the Ca^{2+}-sensing receptor (*J Clin Endocrinol Metab *1996;81[6]:2030-2035).*

The current report is of interest because of the severity of the hypocalcemic symptoms in some of these patients. In previous subjects, hypocalcemia has been modest and the patients often mildly symptomatic or asymptomatic.

Allen W. Root, MD

Protein Turnover During Puberty in Normal Children

Arslanian and Kalhan performed leucine turnover studies in 20 prepubertal Tanner I and 21 pubertal Tanner II through IV nondiabetic children and adolescents. The aim of their study was to determine whether the insulin resistance of puberty involves protein metabolism. Leucine flux, oxidation, and nonoxidative disposal were measured during a primed constant infusion of [1-^{13}C] leucine at baseline and during a stepwise hyperinsulinemic (10 and 40 mU/m^2/min) euglycemic clamp. Indirect calorimetry was performed as well. Breath samples were collected every 5 minutes for the analysis of C13 enrichment in the expired CO_2, and continuous indirect calorimetry by ventilated hood system was used to measure CO_2 production and energy expenditure. During the hyperinsulinemic-euglycemic periods, the glucose was clamped at approximately 100 mg/dL, and arterial blood was sampled every 10 to 15 minutes for determination of isotopic enrichment of plasma ketoisocaproate, amino acids, and insulin.

Fasting plasma glucose and insulin concentrations were similar in both groups, as were leucine and other branched-chain amino acids. Whole body leucine flux, an indicator of proteolysis, was lower in the pubertal versus prepubertal subjects. Similarly, leucine oxidation was lower in pubertal than prepubertal subjects, while nonoxidative leucine disposal (an indicator of protein synthesis) did not differ between the 2 groups. There were no gender-related differences in leucine kinetics. Resting energy expenditure correlated positively with leucine turnover, oxidation, and nonoxidative disposal.

IGF-1 correlated negatively with whole body leucine flux and nonoxidative disposal. Fasting insulin correlated negatively with leucine oxidation but not with leucine flux and nonoxidative leucine disposal.

During the hyperinsulinemic-euglycemic clamp, leucine flux was suppressed from baseline and the suppression was significantly lower in pubertal than in nonpubertal subjects.

The authors conclude that whole body proteolysis is approximately 12% lower in pubertal adolescents compared with prepubertal children, and protein oxidation is 24% lower; however, protein synthesis is similar. They state that this is the first study to demonstrate changes in protein turnover during puberty compared with prepuberty. Protein turnover explained 24% of the variability in resting metabolic rate in these children. They note that the positive correlations between resting energy expenditure and leucine kinetics support the notion that protein turnover is a significant regulator of resting metabolic rate. They also note the inverse relationship between IGF-1 levels and leucine turnover, ie, the higher the IGF-1 level the lower the rate of proteolysis. In addition, studies with the hyperinsulinemic clamp show that pubertal adolescents demonstrate lower levels of proteolysis suppression.

Arslanian SA, Kalhan SC. *Am J Physiol* 1996;270:E79-E84.

Editor's comment: *This is an important and carefully conducted study that significantly advances the understanding of some factors associated with growth during adolescence. The data suggest that (1) puberty is characterized by reduced protein breakdown; (2) pubertal elevations in IGF-1 may play a role in suppressing postabsorptive proteolysis; (3) approximately 20% of resting energy expenditure can be attributed to protein turnover; and (4) during puberty, whole body proteolysis is resistant to suppression by insulin. They carefully point out how their data differ from those collected by others.*

Importantly, the authors point out that this study was done in the postabsorptive state and, therefore, conclusions with regard to postprandial metabolism cannot be extrapolated from their data. It is hoped that such data will be forthcoming, although such studies are significantly more complex to perform and their data are significantly more complex to analyze. Arslanian and Kalhan have substantially increased our knowledge with regard to the events that contribute to growth during adolescence.

William L. Clarke, MD

Morphogenesis and Tumors "Patched" Together in Gorlin Syndrome

Discoveries related to rare genetic syndromes also may provide insight into common diseases. A case in point is the recent delineation of the molecular defect in Gorlin syndrome, or nevoid basal cell carcinoma syndrome (NBCCS). A predisposition to basal cell carcinoma, medulloblastoma, and ovarian fibroma occurs in this autosomal dominant condition, as do diverse malformations involving the ribs, craniofacial structures, digits, and spine. Many of these manifestations reflect localized overgrowth. The underlying defect turns out to be in a gene called *patched (PTC)*, which was studied first in fruit flies as an important developmental control gene. A similar defect may be involved in the most common human cancer, basal cell carcinoma of the skin.

Two teams connected NBCCS to *PTC*. Johnson et al[1] started with their work on the fly gene. When they cloned and mapped human *PTC*, they discovered that it resided very close to or where NBCCS had been mapped. Subsequent analysis in 2 families with NBCCS revealed *PTC* mutations. One was a 9-bp insertion; the other was an 11-bp deletion. They also found a point mutation in a basal cell carcinoma not associated with NBCCS.

Hahn and colleagues[2] used positional cloning to identify *PTC* as the NBCCS gene. Mutations predicted to inactivate *PTC* were found in 6 unrelated NBCCS patients and in tumors from 2 non-NBCCS patients.

Both papers,[1,2] as well as related editorials,[3,4] discussed the *PTC* gene product's normal function and its possible role in the pathogenesis of NBCCS and sporadic basal cell carcinoma. In flies, and presumably in humans, *PTC* encodes a transmembrane glycoprotein that acts as an antagonist in the Hedgehog signaling pathway; it influences the effects of a number of growth factors and morphogens, such as members of the transforming growth factor-β and BMP families, on early embryologic development. Given the inactivating nature of the mutations and the occurrence of tumors in NBCCS, *PTC* must also function as a tumor suppressor gene.

Hahn et al[2] and Shilo[3] speculated that 3 sets of features in NBCCS can be explained by a 2-step mechanism. The first step is the inherited mutation that causes constitutional loss of function at one *PTC* allele, haploinsufficiency; the second step is a sporadic mutation that leads to loss of function at the second allele. They postulated that symmetrical defects, such as craniofacial and overgrowth defects, result from disruption of dosage-sensitive pathways involving *PTC* during early development. Manifestations that are found in random clusters, ie, rib and spine malformations, may reflect sporadic mutations at the second allele in progenitor cells that contribute populations of cells to relevant tissues. Such tissues would be mosaic with regard to *PTC* alleles. Finally, loss of function at the second allele in adulthood leads to basal cell carcinoma and other tumors.

1. Johnson RL, et al. *Science* 1996; 272:1668-1671.
2. Hahn H, et al. *Cell* 1996; 85:841-851.
3. Shilo B-Z. *Nature* 1996; 382:115-116.
4. Pennisi E. *Science* 1996; 272:1583-1584.

Editor's comment: *The authors of all of these reports acknowledge that precisely how* PTC *acts to influence the Hedgehog signaling pathway and how this pathway works in humans is poorly understood. Nevertheless, it seems clear that* PTC *influences the proliferation and perhaps survival of cells during development, growth, and carcinogenesis given the clinical manifestations of NBCCS.*

William A. Horton, MD

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Volume 12, Number 2

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